The Journal of
Thoracic and
Cardiovascular Surgery

VOLUME 40
NOVEMBER 1960
NUMBER 5

Original Communications

THE SUBCORONARY IMPLANTATION OF A FLEXIBLE TRICUSPID AORTIC VALVE PROSTHESIS

Benson B. Roe, M.D., Mervyn F. Burke, M.D. (by invitation), and Harry Zehner, Jr., M.D. (by invitation), San Francisco, Calif.

TWO YEARS AGO we reported to this Association that a flexible plastic tricuspid aortic valve prosthesis had undergone successful testing in animals by long-term implantation in the ascending aorta.[1-4] Three dogs in which these valves have been implanted are still living and angiograms have shown the prosthetic valve to be still competent. When the anatomic valve was made incompetent in these animals they did not survive. It was evident that the subcoronary position of the valve was necessary for coronary filling during diastole and that the usefulness of this valve would be dependent upon the development of a technique for placing it there.

Several improvements in the design, material, and production of this valve have since been effected. While maintaining the basic characteristics of the cusps, we have incorporated new features which have made the valve suitable for intracardiac implantation in the subcoronary position. The current model (Fig. 1) is constructed so that it has the shortest possible axial length which is still adequate to support the valve cusps. Since the septal leaflet of the mitral valve forms the medial wall of the left ventricular outflow tract (see Fig. 5, *A*), it is essential that the prosthesis not protrude into the ventricle. Obviously the distal edge of the prosthesis must be proximal to the ostia of the coronary arteries. Unfortunately the distance between these two structures is only a

From the Surgical Research Laboratories of the Department of Surgery, and the Cardiovascular Research Institute of the University of California School of Medicine, San Francisco 22, Calif.

Supported by grants from the Sacramento (California) Heart Association and the U. S. Public Health Service (H-4680).

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

few millimeters and provides the greatest limiting factor to prosthetic implantation. Additional shortening of the valve jacket in the critical area beneath the coronary ostia has been accomplished by scalloping the distal edge (Fig. 1).

Since the elastomer (silicone) material of which this valve is constructed has proved to be resistant to clotting and tissue reaction, its fixation was dependent upon sutures and friction in the formerly reported technique. In the subcoronary position, however, the valve will not be held by the surrounding

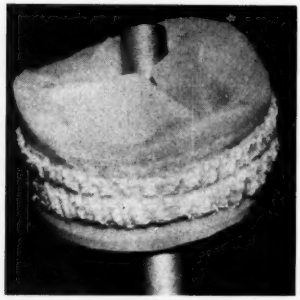


Fig. 1.—Photograph of the smooth-surfaced flexible valve. A single cusp is retracted in the open position. Note the cut-away in the cylinder between the commissures which provides a margin for the coronary ostia. Note also the compressed Ivalon ring which has protected contact with the endothelium.

Fig. 2.—Plaster casting of left ventricular outflow tract and aorta in a dog. Note that there is no ring in the valve area.

tissues because of the irregularly tapering contour of the outflow tract (Fig. 2) and the proximity of the highly flexible mitral valve leaflet. For this reason it has been essential to incorporate two additional features in the jacket: (1) a ring of silicone rubber with greater hardness through which sutures are less likely to tear, and (2) a ring of Ivalon sponge bonded to the silicone which we hope will be invaded by fibrous tissue for fixation if the sutures fail to hold (Fig. 3).

TECHNIQUE OF IMPLANTATION

Technical difficulties were encountered in our initial attempts to suture this valve into place precisely in the area described so that neither the coronary ostia nor the mitral valve was obstructed. Surgical exposure of the full circumference of the aortic "root" is limited and awkward at best, particularly through a vertical aortotomy. The placing of sutures was nearly impossible by

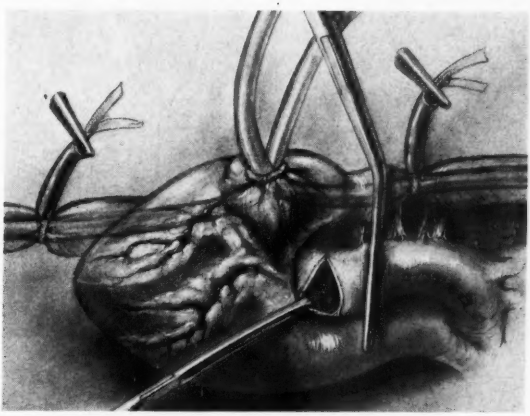
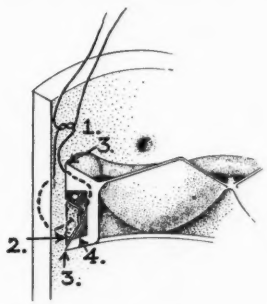


Fig. 3.—Diagram of sagittal section through valve prosthesis which shows position of suture, location of hard plastic band, and Ivalon sponge "jacket." Note flared edges for tight surface contact.



1. Knot hidden behind upper lip of valve
2. Ivalon covered above and below
3. Feathered edges to prevent clot
4. Hard silastic to hold suture

Fig. 4.—Diagram of operative approach through a right thoracotomy.

ordinary methods because of the difficulty in directing needles vertically. The present technique, however, has largely overcome these obstacles and it is now possible to insert the valve in the subcoronary position through a transverse aortotomy (Fig. 4) with seven precisely placed sutures in a period which seldom exceeds 30 minutes of aortic cross-clamping. Myocardial ischemia is reduced

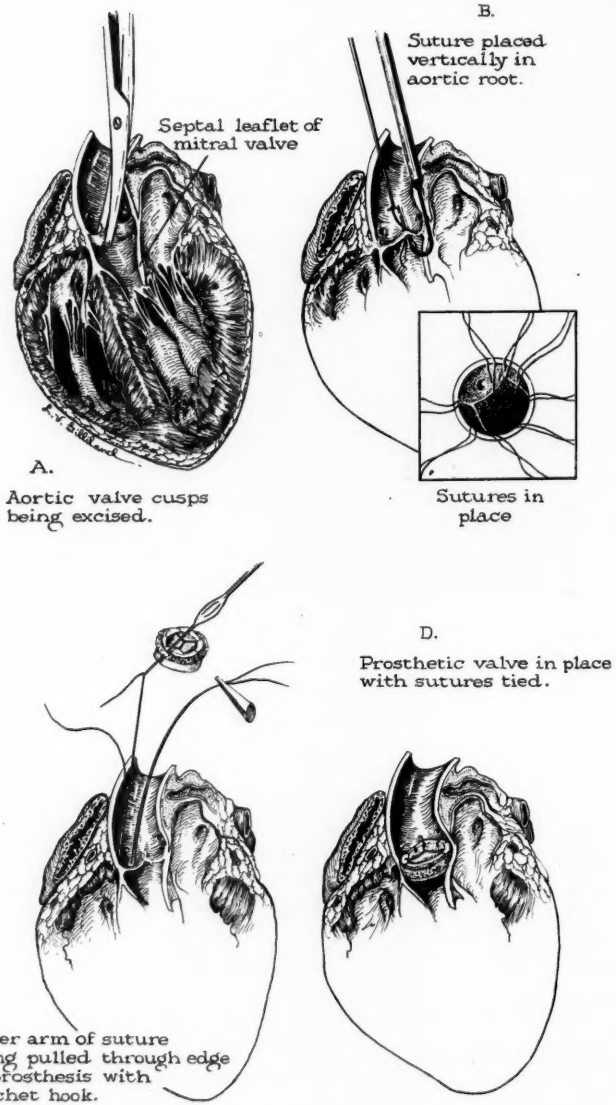

B.
Suture placed vertically in aortic root.
Septal leaflet of mitral valve
A.
Aortic valve cusps being excised.
Sutures in place
D.
Prosthetic valve in place with sutures tied.
C.
Lower arm of suture being pulled through edge of prosthesis with crochet hook.

Fig. 5.—Diagrams of suturing technique.

A, Resection of remaining anatomic cusps.

B, Introduction of "J" needle from below upward through the valve remnant. The free end of the suture is drawn through the tissue and fed out from a spool through the hollow needle. The needle is then withdrawn to deliver the lower arm of the suture.

C, The crochet hook is inserted through the edge of the prosthetic valve and draws the bottom arm of the suture through the plastic material.

D, Prosthetic valve in place with sutures tied.

by producing regional (cardiac) deep hypothermia and by additional intermittent perfusion of the coronary ostia with cold blood during the procedure.

To permit accurate placing of sutures it is necessary to excise the anatomic cusps of the diseased valve (or the normal cusps of the valve in the experimental animal) (Fig. 5, *A*). The sutures necessary for holding the valve to the aortic wall must be placed in an axial direction parallel to each other and at the same level below the coronary ostia. This maneuver is accomplished under direct vision with a specially developed hollow "J" needle which has been described elsewhere[5] (Fig. 5, *B*). One suture is placed in each of the three commissures and one or more sutures in each cusp remnant, according to the size of the valve.

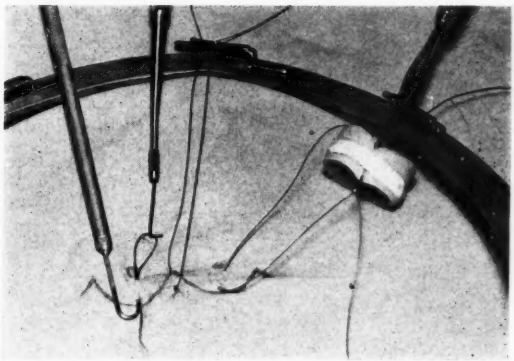


Fig. 6.—Suture technique demonstrated with instruments and simulated valve. Note superimposed clips to maintain sutures in alignment on the flat metal ring which surrounds the operative field.

To prevent tangling, the multiple suture ends are held in clips on a metal ring which surrounds the operative field (Fig. 6). Twisting is also avoided and the lower limb of the suture is quickly identified in the top clip when the suture is later passed through the prosthesis.

After all the sutures have been placed in the aortic wall, a crochet hook is used to draw the lower limb of each suture through the wall of the prosthetic valve around the hardened ring in an appropriate position on the prosthetic cuff to match the location of the suture on the aortic wall (Fig. 5, *C*). This step is accomplished in such a way that the suture holes and knots are covered behind the valve jacket. The valve is then slid down the sutures into position and the sutures are tied (Fig. 5, *D*). The aortotomy is then closed.

RESULTS

In early attempts to implant a prosthesis in the subcoronary location, the incidence of technical failure was high but several animals survived long enough to demonstrate that the valve worked. Since the development of the technique described, we have operated on 21 animals with extracorporeal circulation and cardiac arrest with regional hypothermia. In every instance the prosthetic

valve was implanted satisfactorily. All but one heart developed ventricular fibrillation on rewarming but one or two defibrillatory shocks resulted in regular effective contractile beat in every animal. In 4 dogs the hearts returned to persistent ventricular fibrillation as the result of unexplained myocardial infarcts developed during the procedure. Three dogs died of uncontrolled bleeding from the aorta when normal circulation had been restored.

The remaining 14 dogs maintained satisfactory vital signs, temporarily supported by vasopressors, for an average period of 8 hours. Their chest incisions were closed, heparinization was neutralized, and spontaneous breathing restored. Death occurred from 2 to 18 hours later, usually with a gradually declining blood pressure or evidence of neurological damage.

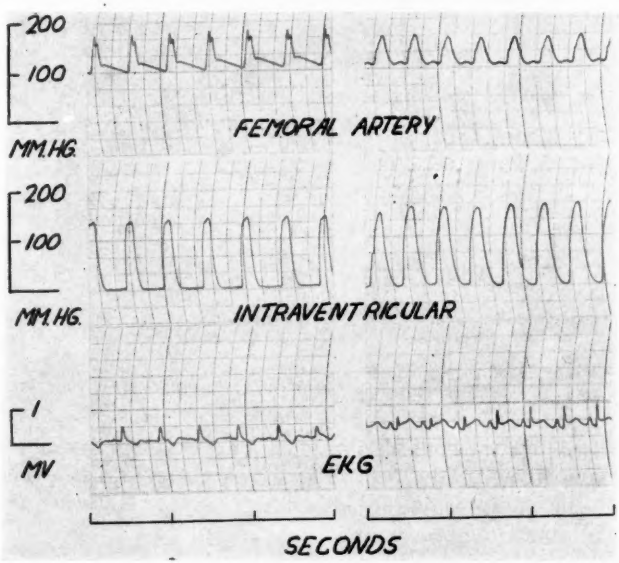


Fig. 7.—Simultaneous pressure curves of normally beating heart above and below prosthetic aortic valve. (Preoperative pressures on the *left* and postoperative on the *right*.) Note dicrotic notch and absence of systolic gradient.

Pressure determinations after implantations (Fig. 7) reveal no systolic gradient across the valve, a dicrotic notch, and a normal systemic diastolic pressure with a zero intraventricular pressure. This evidence supports the observation of valve function in a pulse duplicator which shows complete competence.

Unfortunately, we have not yet been successful in maintaining long-term survivors to determine the eventual fate of this prosthetic valve, but the clinical course and the physiologic data on these animals suggest that death was the result of either prolonged cardiac ischemia or was due to our extracorporeal perfusion technique, rather than to the presence of the prosthesis in the heart.

Autopsy examinations have revealed no evidence of obstruction to the coronary ostia in this series. There has been no clot formation on the valve and

no avulsion of sutures. Small to moderate-sized hematomas have been found in the subserosal layer of the mitral valve leaflet and in the interatrial septum. Large hematomas were sometimes found also in the periaortic fat pad and in the atrioventricular groove. The occurrence of all these hematomas appears to be related more to the aortotomy closure than to valve placement since they also appeared in the hearts of animals subjected to control procedures without valve implantation.

SUMMARY

A technique has been described for implantation of a short flexible aortic valve prosthesis in the subcoronary position under direct vision in the experimental animal. It has been shown that normal hemodynamics can be restored by this procedure after total ablation of the anatomic valve. Coronary blood flow is not obstructed. Previous long-term studies indicate that the physical characteristics of the valve satisfactorily prevent clotting and maintain function. Long-term survivors are still needed to determine whether permanent fixation can be expected from this technique.

REFERENCES

1. Roe, B. B., Owsley, J. W., and Boudoures, P. C.: Experimental Results With a Prosthetic Aortic Valve, J. THORACIC SURG. 36: 563-570, 1958.
2. Roe, B. B., and Moore, D.: Design and Fabrication of Prosthetic Valves, Exper. Med. & Surg. 16: 177-182, 1958.
3. Roe, B. B.: A Simple Device to Facilitate Coronary Artery Perfusion, Surgery 44: 554-555, 1958.
4. Roe, B. B., Najarian, J. S., and Moore, M.: A Technic for Insertion of a Flexible Aortic Valve Prosthesis Into the Ascending Aorta, Ann. Surg. 145: 388-391, 1957.
5. Roe, B. B.: A Simplified Technique for Closure of Interventricular Septal Defects, J. THORACIC SURG. 40: 232, 1960.

(For Discussion, see page 631)

EXPERIMENTAL PRODUCTION OF AORTIC STENOSIS

Anthony J. Munoz, M.D. (by invitation), and Sam E. Stephenson, Jr., M.D. (by invitation), Nashville, Tenn.

THE PRODUCTION of lesions simulating acquired human cardiac lesions presents a major problem in laboratory animals. Previous work directed at the production of experimental aortic stenosis has utilized the application of a cannula,[1] metal clips,[2] or sutures[3] in the commissures. These experiments have afforded unpredictable and inconstant degrees of aortic stenosis. In an attempt to produce coronary arteriosclerosis in the experimental animal it was noted that the first site of plaque formation in the animals was one encircling the aortic annulus and, in some cases, fusion of the commissures developed. This then represents the early lesion of aortic stenosis.

METHOD

Unselected mongrel male dogs, weighing from 11 to 23 kilograms, were utilized. After a baseline electrocardiogram and serum cholesterol levels were obtained, the animals were given 2 millicuries per pound of I^{131} to render the dogs euthyroid or hypothyroid. (In a second group of animals [Group B], propylthiouracil, 750 to 1,000 mg. per day, was substituted for the radioactive iodine.) After a 2-week delay the animals were started on 100,000 I.U. of vitamin D daily and placed on a special diet (Table I). Fresh meat was added at frequent intervals.

TABLE I*

A.	
Commercial dog meal	93 grams
Commercial lard	5 grams
Crystalline cholesterol	2 grams
B.	
Cooking oil	73.0 grams/100
Cholesterol	20.0
Cholic acid	5.0
Thiouracil	2.0

*Diet A was utilized in combination with I^{131} and diet B when thiourea derivative was substituted.

From the S. R. Light Laboratory for Surgical Research, Department of Surgery, Vanderbilt University School of Medicine, Nashville, Tenn.

Aided in Part by U. S. Public Health Service Grant 3047.

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

The vitamin D was continued until the dogs demonstrated the toxic manifestations of weight loss and bleeding from the gums and it was then reduced by one half. The special diet was continued until sacrifice. Monthly serum cholesterol levels were obtained (Table II). At the time of sacrifice some animals had coronary arteriograms and others had left heart catheterization for the gradient across the aortic valve.

TABLE II*

A. I^{131}	+ Diet	+ Vitamin D
	Avg. Mg./%	*Range*
Preop.	154	125-180
1 mo.	833	450-1,120
2 mo.	965	620-1,170
3 mo.	1,094	775-1,360
B. Thiouracil	**+ Diet**	**+ Vitamin D**
	Avg. Mg./%	*Range*
Preop.	171	115-235
1 mo.	159	105-195
2 mo.	303	225-355
3 mo.	371	235-490
4 mo.	638	575-740
5 mo.	605	500-770
6 mo.	940	660-1,450

*The group A animals show the more rapid rise in serum cholesterol levels achieved with this method and the lesions were more prominent in this particular group.

The excised heart and great vessels were examined, photographed, and preserved in formalin solution. In some cases the entire heart and great vessels were stained with Sudan III for localization of fatty deposits not grossly visible. Typical areas of plaque formation were submitted for microscopic examination.

RESULTS

Six animals were prepared, using radioactive iodine, vitamin D, and the high cholesterol diet. All animals were sacrificed at 3 months and all showed marked atherosclerosis encircling the aortic annulus (Fig. 1). In some animals there were also marked changes in the pulmonary artery, arch of the aorta, descending aorta, and abdominal aorta. These areas of involvement grossly resembled human plaques, both in their appearance and location. The location of these plaques was primarily on the superior wall of the thoracic aorta, around the orifices of the branch vessels, and on the posterior wall of the abdominal aorta. Unfortunately pressure gradients were not obtained on these animals as the finding of commissure fusion was unexpected.

Four additional animals were prepared, but propylthiouracil rather than I^{131} was given to suppress thyroid function. Although these animals also responded with a high serum cholesterol level, the findings were not as consistent and plaque formation not as prominent. These animals were sacrificed at the end of 6 months and demonstrated an average gradient of 49 mm. Hg across the aortic valve.

The serum cholesterol values on these animals prepared with radioactive iodine averaged 1,094 mg. % at 3 months. It is possible, however, with this regimen to obtain cholesterol values in excess of 1,800 mg. %. In reviewing these groups of animals it seems that levels in excess of 600 to 800 mg. % need to be maintained for 3 consecutive months and then satisfactory plaque formation will have occurred.

It must be stated that the lesions observed in these animals have not progressed as yet to the full blown "pile of rocks" so classically seen in clinical calcific aortic stenosis. These lesions have been early lesions suggestive of aortic stenosis, both to us and to the pathologist. The gross lesions have appeared as deposits with or without calcification encircling the annulus, fusion of the commissures, and fatty deposition on the leaflets, the latter demonstrated by supravital staining of the gross specimen with Sudan III.

Fig. 1.—Photograph of typical specimen from animal sacrificed at 3 months. Commissure fusion is not as marked in this specimen as in some but it also demonstrates the lesions in the arch, pulmonary artery, and coronary arteries.

Attempts to demonstrate this degree of aortic stenosis by arteriographic means have not been satisfactory. Demonstration of the nicking from plaque formation in the main and branch coronary arteries has been possible by radiographic study prior to sacrifice.

Although the gross examination of these specimens resembles the plaques seen in human arteriosclerosis, the microscopic appearance is somewhat different and thus not completely analogous. We would like to reproduce the human counterpart of the disease but these experimental lesions offer a means heretofore unavailable for the study of the early changes of aortic stenosis, coronary sclerosis, and partial occlusion (by plaque formation and narrowing) of the small branch vessels.

The microscopic picture is primarily one of subintimal hemorrhage and fatty deposits in the early stages. These changes are soon followed by an increase in fibrous tissue, large fatty deposits, and, later, marked calcification of these areas (Fig. 2) with elevation of the intima, progressing in some cases to actual intimal ulceration. For the most part these changes are similar to the lesion in medial sclerosis.

DISCUSSION

The early work of Creech and associates[4] pointed out the feasibility of the development of arteriosclerotic-like plaques in dogs. Up until that time this lesion had been extremely difficult to produce. Soon after the reported

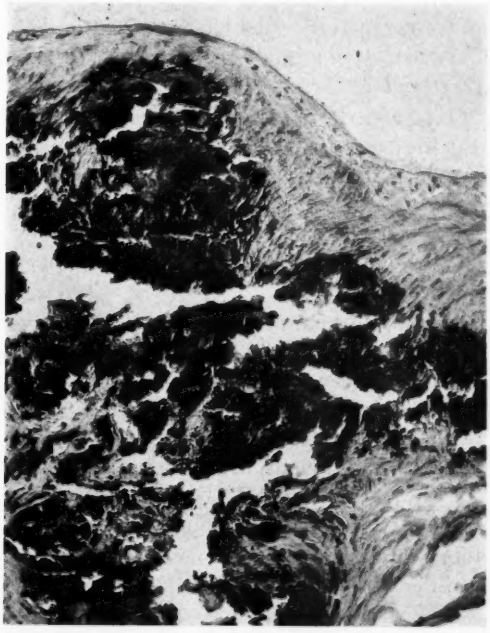


Fig. 2.—Photomicrograph of lesion at the aortic annulus. This animal had been treated for 90 days. One can see the marked calcification and numerous cholesterol clefts. (×25; reduced ⅛.)

work of Creech, we set up a long-term study, using the Tulane method of producing arteriosclerotic disease, in an attempt to assess mechanical factors concerned in the selective deposition of the material. Through a period of 3 years we have been able to produce some lesions. These, however, have not been of the magnitude expected and the long period of treatment is not only exhausting to the animal and the research group but the cost is exorbitant. It is possible, however, to produce changes in the abdominal aorta by this method and this process is enhanced by mechanical irritation of the vessels.

Blake,[5] using a variant of this method, was able to develop similar lesions in dogs. In these studies, coronary lesions developed in somewhat over 24 months of treatment.

Crisp[6] in 1958 reported the fairly rapid development of lesions in dogs by another method. Utilizing regular dog chow as a diet the animals were given 100,000 I.U. of vitamin D daily. After about 90 days, lesions in the media of major vessels were evident.

The procedure utilized in this report is essentially a combination of these two methods. The vitamin D in such large doses produces multiple subintimal hemorrhages. These areas of injury then predispose to the deposition of the excess lipids in these particular areas. Although the lesions produced by this method are not completely analogous to the human lesions of arteriosclerosis, the sites of deposition are the same. The experimental lesions select, in sites of decreasing frequency, the aortic annulus, the superior wall of the ascending aorta, the pulmonary artery, the lumbar portion of the abdominal aorta, and, last, the coronary arteries.

It would be preferable to be able to develop lesions in animals which are microscopically the same as those in human beings. As yet this is not possible. Experiments are now in progress using primates with the same technique in an effort to evaluate this type of experimental animal. Until it is possible to develop the human type lesions, the method herein described appears to represent more closely the human counterpart than the lesions from previously described methods.

The utilization of propylthiouracil instead of radioactive iodine, for preparation of the animals before vitamin D and cholesterol are started, produces similar lesions but these lesions are not as marked, and the degree of fusion of the aortic commissures is inconstant. At the present time we consider that I^{131} should be used to produce the more marked results. It must be remembered, however, that if the animals are kept under observation for longer than 6 months, repeat treatment with I^{131} may be necessary.

It is hoped that animals prepared over a longer length of time will show a more complete and advanced aortic stenosis. Many other aspects of this over-all problem need to be evaluated and will form the basis of a subsequent report.

SUMMARY

1. A method is described to produce arteriosclerotic-like lesions in male dogs. These lesions develop in less than 90 days.

2. Among the earliest lesions are those encircling the aortic annulus and, if permitted to progress, cause fusion of the aortic commissures and the early lesions of aortic stenosis.

3. The use of I^{131}, 2 millicuries per pound of animal weight, to suppress thyroid function is superior to the utilization of antithyroid drugs for the consistent development of the lesions in this particular experiment.

REFERENCES

1. Meerson, F. Z., and Kobozev, G. W.: O methodike sozdanica eksperimentalnogo stenoza aorty, Bulletin Eksperimental'noi Biologic i meditsiny (Moskow) **39**: 50-52, 1955.
2. Salemo, R. A.: The Experimental Production of Valvular Aortic Stenosis, Ann. Surg. **149**: 368, 1959.
3. Boerma, J. R., Germs, J., and Blickman, J. R.: Artificial Stenosis of the Aorta, Chirurgicum Neerlandicum **3**: 123, 1951.
4. Creech, O. J., Jordan, G. L., Jr., De Bakey, M. E., Overton, R. C., and Halpert, B.: The Effect of Chronic Hypercholesterolemia on Canine Aortic Transplants, Surg. Gynec. & Obst. **101**: 607, 1955.
5. Blake, T. M.: Personal communication, 1960.
6. Crisp, W. W., Jr., Root, H. D., Jenson, C. B., Imamagler, K., and Yonehiro, E. G.: Experimental Production of Arteriosclerosis in the Dog, S. Forum **9**: 304, 1958.

(For Discussion, see page 631)

EVALUATION OF NEW NONWETTABLE MACROPOROUS MEMBRANES WITH HIGH PERMEABILITY COEFFICIENTS FOR POSSIBLE USE IN A MEMBRANE OXYGENATOR

James S. McCaughan, M.D. (by invitation), Richard Weeder, B.A. (by invitation), John C. Schuder, Ph.D. (by invitation), and William S. Blakemore, M.D., Philadelphia, Pa.

A MEMBRANE that is permeable enough to oxygenate blood adequately without requiring a large surface area or volume of blood would be a desirable contribution in the development of a practical membrane oxygenator. Various thicknesses of different membranes have been evaluated empirically in oxygenators.[5, 7, 8, 18] Since blood must be circulated through an oxygenator and the increase in the oxygen saturation determined, these experiments have been expensive, arduous, and difficult to control.

In his experiments on the diffusion of gases through solids, Barrer[2, 4] showed that the gas first dissolves in the membrane, diffuses across the membrane, and then evaporates into the atmosphere on the other side. The permeability of the membrane is thus determined by the physical and chemical structure of the membrane and the nature of the gas diffusing through it. Except for a few anomalous systems,[12] the flow rate of a gas through the membrane can then be (1) increased linearly by increasing the partial pressure difference of the gas across the membrane, (2) decreased linearly by increasing the thickness of the membrane, and (3) increased logarithmically by increasing the absolute temperature.[1, 3, 6, 13]

From these observations, Barrer developed the concept of a permeability coefficient which is equal to the product of the solubility and diffusion constants of the gas and is defined as the standard cubic centimeters (0° C. 1 atm.) of gas passing through 1 sq. cm. of membrane (1 mm. thick) per second per centimeter of mercury partial pressure difference across the membrane.*

From the Harrison Department of Surgical Research and the Department of Surgery of the University of Pennsylvania.

Supported by a grant from the Southeastern Pennsylvania Chapter of the American Heart Association.

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

*Thus, the following mathematical expression can be derived for the flow of a gas across a polymer membrane:

$$\frac{\text{Flow rate}}{\text{Unit area}} = \frac{\text{Permeability coefficient} \times \text{Partial pressure difference}}{\text{Membrane thickness}}$$

or

$$\frac{V}{A} = \frac{P\ (p_1 - p_2)}{t}$$

where

V = flow rate of gas across the membrane (c.c./sec.).
A = area of membrane exposed (sq. cm.).
p_1 = partial pressure of the gas on one side of the membrane (cm. Hg).
p_2 = partial pressure of the gas on the other side of the membrane (cm. Hg).
t = thickness of the membrane (mm.)
P = permeability coefficient for the given gas-membrane system at a specific temperature (c.c.-mm./cm.²-cm. Hg-sec.).

By measuring the flow rate of a gas across different membranes under controlled conditions, the permeability coefficients can be calculated for those specific gas-membrane systems. From a comparison of the permeability coefficients, the most permeable membrane can then be selected.

Various experimental procedures have been used to measure these coefficients; however, they have usually been either elaborate or time consuming.[4, 5, 9, 11, 16, 18, 20, 22, 23] Although the permeability coefficients for oxygen and carbon dioxide have been determined for almost all the membranes currently available, we undertook to develop a simple technique for measuring the permeability coefficients of several new types of membranes. With this apparatus, rapid and easy preliminary studies can be performed to measure the relative permeabilities of different membranes without the necessity of using an oxygenator with all the problems it entails.

Because permeation is a solution and diffusion phenomenon, two gases will permeate different membranes with approximately the same relative rates which is dependent upon the molecular weight of a gas (Graham's law).[25] Therefore, the membranes that have the highest permeability to carbon monoxide will also have the highest permeability to oxygen. Since a sensitive carbon monoxide analyzer was available and we are interested in the permeabilities of different membranes relative to each other, we measured the carbon monoxide permeability of various standard membranes to establish a base line for future comparison. In the technique that will be described, the permeability of any gas can be determined by analyzing the gas mixtures for the specific gas with the appropriate analyzer.

PRINCIPLE

When pure carbon monoxide is passed on one side of a membrane and oxygen on the other side, some of the gases will counter diffuse through the membrane. The gas mixture leaving the oxygen side of the chamber can then be analyzed for carbon monoxide content. Since this represents the carbon monoxide that diffused through the membrane, the permeability of the membrane to carbon monoxide can be calculated (Fig. 1).

From a carbon monoxide mass balance, the perfect gas law, and the flow equation, a relationship can be derived for calculating the carbon monoxide permeability constant from the data obtained with this apparatus.*

APPARATUS

1. *Diffusion Chamber.*—This consists of two 3.5 by 3.5 by 1 in. thick hollow aluminum plates which are bolted together with a membrane secured between

$$*P = \frac{(273)}{(T + 273)} \frac{(P_O + p_D)}{76} \left(\frac{x_D V_D t}{A(P_O + p_A)} \right)$$

where T = chamber temperature (°C.).
P_O = atmospheric pressure (cm. Hg).
p_D = pressure in exhaust line from oxygen side of chamber (cm. Hg).
p_A = pressure in inflow line to CO side of chamber (cm. Hg).
V_D = flow rate of exhaust gas from oxygen side of chamber (c.c./sec.).
A = surface area of the membrane (sq. cm.).
t = membrane thickness (mm.).
x_D = mol fraction of CO in exhaust gas from oxygen side of chamber.
P = permeability coefficient for CO at temperature T (c.c.-mm./sec. cm.^{2}-cm. Hg) referred to STP conditions.

them by 2⅛ inch diameter rubber "O" rings (Figs. 2 and 3). Each plate has a tube for the entrance and exit of a gas and a thermometer well. A wire screen is placed inside of each "O" ring to prevent the membrane from buckling.

2. *Manometers.*—The pressure of each entrance and exhaust gas is measured with a mercury manometer.

3. *Flowmeter.*—The flow rate of the gas in the oxygen exhaust line is measured with a 1,000 c.c. per minute capacity rotameter† and the flow rates of the carbon monoxide entrance and exhaust gases are monitored with 45 c.c. per minute capacity "Tri-Flat" flowmeters.*

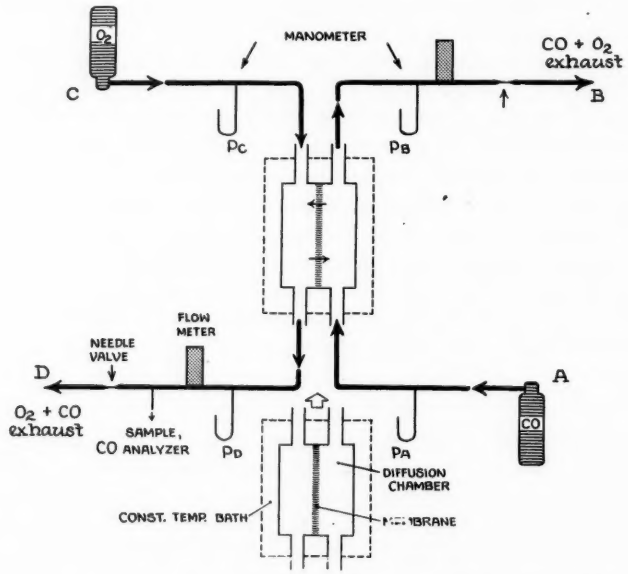


Fig. 1.—Diagram of technique used for measuring the permeability coefficients of membranes.

4. *Temperature Control.*—The entire diffusion chamber is immersed in a water bath which maintained the temperature at 30 ± 0.5° C.

5. *Gas Analysis.*—The gas leaving the oxygen side of the chamber is analyzed with a Stalex† carbon monoxide analyzer for the amount of carbon monoxide present in the sample.

6. *Membrane Thickness.*—The thickness of each piece of membrane is measured with a dial micrometer.‡ This is important since there is often a size variation in the actual thickness of a membrane than that listed by the manufacturer. Also, the thickness of many of the membranes is not uniform.

*Fisher and Porter Co., Hatboro, Pennsylvania.
†Model SI-2, Stalex Corporation, Stockholm, Sweden.
‡Model 55412, B. C. Ames Co., Waltham, Mass.

PROCEDURE

After the thickness has been measured, a 7 cm. by 7 cm. piece of the membrane is placed in the diffusion chamber between the rubber "O" rings and the chamber is bolted together and immersed in the constant temperature bath.

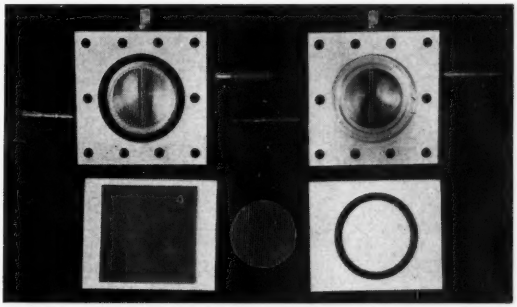


Fig. 2.—Diffusion chamber disassembled.

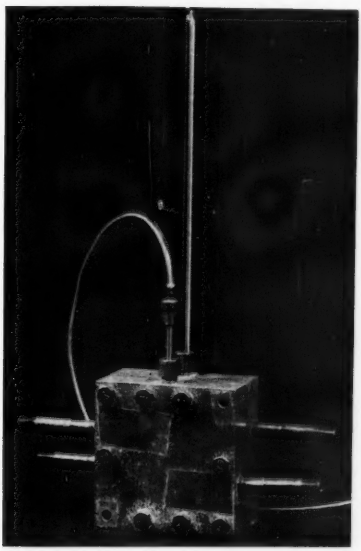


Fig. 3.—Diffusion chamber assembled with thermometer and thermocouple in place.

The temperature in the chamber is controlled at 30° ± 0.5° C. by a thermocouple placed in one of the thermometer wells. Oxygen is then slowly passed through one side of the chamber and carbon monoxide through the other. The needle valves on the exhaust lines are then simultaneously adjusted so the pressures on both sides of the membrane gradually rise to approximately the same value. Thus, the membrane is prevented from rupturing by keeping the total

pressure gradient across the membrane negligible, although the partial pressure difference of each gas across the membrane is greater than one atmosphere.

After steady conditions have been obtained, recordings are made of the temperature of the chamber, the pressure in all of the four gas lines, and the flow rate of the gas leaving the oxygen side of the chamber. A sample of this exhaust gas is then removed with a needle and syringe and injected into the carbon monoxide analyzer which automatically records the amount of carbon monoxide present in the sample.[15] Thus, an entire experiment can be performed in less than 30 minutes.

SAMPLE DATA AND CALCULATIONS

The following data illustrate the reproducibility of the results:

Membrane: Cast Teflon	*Sample 1**	*Sample 2*	*Sample 3*
Thickness (t) (mm.)	.0127	.0063	.0127
Temperature (T) (°C.)	30.3	29.5	29.7
Pressure of oxygen exhaust (p_D) (cm. Hg)	20.0	19.5	20.0
Pressure of CO inflow (p_A) (cm. Hg)	20.0	19.5	20.0
Flow rate of oxygen exhaust (V_D) (c.c./sec.)	8.53	3.33	3.70
Surface area of membrane (A) (sq. cm.)	28.18	28.18	28.18
Mol fraction of CO in oxygen exhaust (x_D)	.000138	.000655	.000319
Atmospheric pressure (P_O (cm. Hg)	75.5	75.5	75.5
Permeability constant (c.c.-mm./sec.-cm. Hg-cm.^{2})	6.31×10^{-9}	5.80×10^{-9}	5.71×10^{-9}

RESULTS AND DISCUSSION

The evaluation of membranes in oxygenators has been expensive and difficult, requiring large sheets of the plastic, animals, blood, etc. These problems have been compounded by testing different thicknesses of the same membrane as though they were different membranes. By using the concept of a permeability coefficient, small pieces of experimental membranes can be tested easily. Since the permeability coefficient is expressed as though all membranes were 1 mm. thick, the necessity for testing different thicknesses of the same membrane is eliminated. However, the thickness of the membranes commercially available must be considered when making a final practical evaluation.

In using a dynamic system, such as described, to measure permeability coefficients, a minimum gas flow is required to prevent streaming and stagnation in various parts of the apparatus. This manifests itself by yielding lower and erratic diffusion rates when the flow is below the minimum value. The carbon monoxide permeability coefficients of several membranes are plotted as a function of gas flow rate in Fig. 4. Above a flow rate of approximately 100 c.c.

*For Sample 1 the calculation was:

$$P = \frac{(273)}{(T + 273)} \left(\frac{P_O + p_D}{76}\right) \left(\frac{x_D V_D\, t}{A(P_O + p_A)}\right).$$

$$P = \frac{(273)}{(273 + 30.3)} \frac{(75.5 + 20.0)}{(76)} \frac{(.000138)\ (8.53)\ (.0127)}{(28.18)\ \ (75.5 + 20.0)}.$$

$$P = 6.31 \times 10^{-9} \frac{\text{c.c.- nm.}}{\text{sec.-cm.}^2\text{-cm. Hg}}.$$

per minute, the permeability coefficient is relatively independent of flow rate. This minimum flow rate is probably a function of the physical design of the apparatus and should be determined for each apparatus.

The permeability coefficients of several previously studied membranes were obtained for comparison with many new types of membranes which were tested. The most promising of these are E. P. Pormax,* N. P. Pormax,* and micropolyethylene.* These "macroporous" membranes are especially prepared by mixing inert molecules, such as starch, with the plastic formula. After the plastic sheets have been prepared, the starch molecules are leached out of the

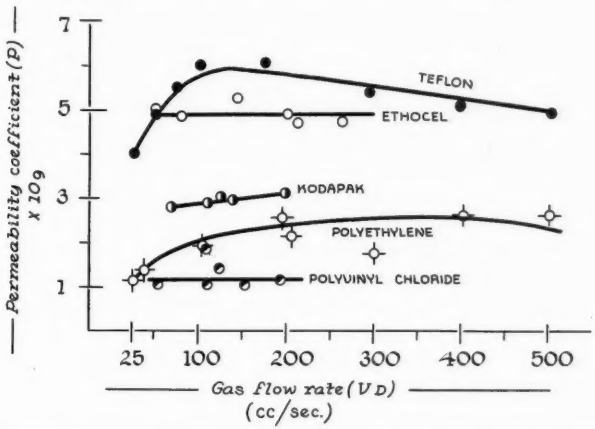


Fig. 4.—Permeability coefficients as a function of gas flow rate as measured with this apparatus.

membranes, thereby leaving tiny "pores" in the membranes. The diameters of the "pores" can be varied from 3 to 300 micra by using inert substances having different molecular sizes. The per cent porosity of the membranes can be varied by altering the per cent of inert material added. These studies have shown the permeability of these new membranes to be more dependent on the pore size and per cent porosity than on the type of plastic used.

Table I lists the carbon monoxide permeability coefficients determined with this apparatus and the coefficients for carbon dioxide obtained from the literature. Since the ratio of the permeability coefficients of two gases is relatively constant for almost all membranes, comparison of the ratios of the carbon monoxide permeability coefficients to those of carbon dioxide and oxygen provides a measure of the reliability of the data.

The permeability data obtained with this simple apparatus and technique warranted the further development of the experimental macroporous membranes and led to its production in larger sizes.

Partial perfusions of dogs with a small prototype oxygenator, using Teflon, Silastic, and N. P. Pormax membranes, were performed. The partial pressure

*Supplied by Dr. Howard J. Strauss, ESB—Reeves Co., Glenside, Pa.

TABLE I*

MEMBRANE	P_{CO}	P_{O_2}	P_{CO_2}	$\frac{P_{CO_2}}{P_{O_2}}$	$\frac{P_{CO}}{P_{O_2}}$	$\frac{P_{CO}}{P_{CO_2}}$
Polyvinyl chloride	1.2	—	12[16]	—	—	0.10
Polyethylene	2.5	4.1[16]	18[16]	4.4	0.6	0.14
Cellulose acetate butyrate (Kodapak II)	3.2	—	35[16]	—	—	0.09
Ethylcellulose (Ethocel)	5.4	10[16]	43[10]	4.3	0.54	0.13
Tetrafluoroethylene (Teflon)	5.8	—	—	—	—	—
Natural rubber	18	32.7[16]	163[16]	5.0	0.55	0.11
Silicone rubber (Silastic S-2000)	375	650[10]	3,400[10]	5.2	0.58	0.11
N. P. Pormax	13,000-16,000	—	—	—	—	—
E. P. Pormax	15,000-25,000	—	—	—	—	—
Micro-Pormax	22,000-40,000	—	—	—	—	—

*Permeability coefficients at 30° C. P. = c.c.-mm./sec.-cm.^{2}-cm. Hg $\times$ 10^9 corrected to STP.

of the oxygen in the blood leaving and entering the animal was continuously monitored with oxygen electrodes.[21] Thus the effects of varying blood and oxygen flow rates and pressures could be noted immediately.

The average oxygen uptake for 0.00045 in. thick Teflon was 15 c.c. per minute per square meter. This compared with the average uptake of approximately 12 c.c. per minute per square meter reported by Pierce.[19] Silastic, 0.0068 in. thick, provided an uptake of 24 c.c. of oxygen per minute per square meter. Using N. P. Pormax with average pores of 8 to 12 micra, the oxygen uptake initially was over 35 c.c. of oxygen per minute per square meter when the oxygen pressure was one atmosphere. Because of the large pore size, however, blood rapidly leaked through the membrane into the oxygen chambers. This was only partially corrected by eliminating all wetting agents from the formulation but blood continued to "ooze" through when the pressure of the blood was greater than 30 mm. Hg. Inherent resistance to flow of blood in the oxygenator demanded a pressure greater than this. By increasing the oxygen pressure, the ooze could be further decreased and the oxygenation greatly increased. However, at a critical pressure, fine bubbles would appear in the blood. Presently new membranes are being prepared with smaller pores for evaluation.

SUMMARY

The advantage of using the concept of a permeability coefficient for indicating the relative permeability of different membranes to gases is described along with a simple apparatus and technique for determining the coefficients. This method permits the rapid screening of new membranes for possible use in a membrane oxygenator without testing different thicknesses of the same membrane or using an oxygenator with all the problems and expense it entails.

The permeability coefficients of several standard membranes and new plastic membranes containing "pores" which can be varied in diameter from 3 to 300 micra are listed and the membranes described.

Preliminary tests in a prototype oxygenator indicate these new membranes have superior oxygenating capacities but need development of membranes with smaller pore sizes.

REFERENCES

1. Van Amerongen, C. J.: Influence of Structure of Elastomers on Their Permeability to Gases, J. Polymer Sc. 5: 307, 1950.
2. Barrer, R. M.: Diffusion In and Through Solids, London, 1941, Cambridge University Press, p. 60.
3. Barrer, R. M.: Diffusion In and Through Solids, London, 1941, Cambridge University Press, p. 403.
4. Barrer, R. M., and Skirrow, G.: Transport and Equilibrium Phenomena in Gas-Elastomer Systems. I. Kinetic Phenomena, J. Polymer Sc. 3: 549, 1948.
5. Benvenuto, R., and Lewis, F. J.: Influence of Some Physical Factors Upon Oxygenation With a Plastic Membrane Oxygenator, Surgery 46: 1099, 1959.
6. Brubaker, D. W., and Kammermeyer, K.: Flow of Gases Through Plastic Membranes, Indust. Eng. Chem. 45: 1148, 1953.
7. Clowes, G. H. A., Neville, W. E., Sabga, G., and Shibota, Y.: The Relationship of Oxygen Consumption, Perfusion Rate, and Temperature to the Acidosis Associated With Cardiopulmonary Circulatory Bypass, Surgery 44: 220, 1958.
8. Clowes, G. H. A., Hopkins, A. L., and Neville, W. E.: An Artificial Lung Dependent Upon Diffusion of Oxygen and Carbon Dioxide Through Plastic Membranes, J. THORACIC SURG. 32: 630, 1956.
9. Doty, P. M., Aiden, W. H., and Mark, H.: Water Vapor Permeability of Organic Films, Indust. Eng. Chem., Anal. Ed. 16: 686, 1944.
10. Dow Corning Corp., Midland, Mich., Personal communication, 1959.
11. Hancher, C. W., and Kammermeyer, K.: Semiautomatic Gas Separation Equipment, Anal. Chem. 27: 83, 1955.
12. Jost, W.: Diffusion in Solids, Liquids, Gases, New York, 1952, Academic Press, N. Y., p. 297.
13. Jost, W.: Diffusion in Solids, Liquids, Gases, New York, 1952, Academic Press, N. Y., p. 285.
14. Kammermeyer, K.: Silicone Rubber as a Selective Barrier, I. E. C. 49: 1685, 1957.
15. Linderholm, H., Danielson, G. K., and Blakemore, W. S.: (To be published.)
16. Osburn, J. O., and Kammermeyer, K.: Separation of Gases by Plastic Membranes, New Diffusion Cell Design, Indust. Eng. Chem. 46: 739, 1954.
17. Othmer, D. F., and Frohlich, G. J.: Correlating Permeability Constants of Gases Through Plastic Membranes, Indust. Eng. Chem. 47: 1034, 1955.
18. Park, W. R. R.: Semimicro Gas Permeability Apparatus for Sheet Material, Anal. Chem. 29: 1897, 1957.
19. Pierce, E. C.: Diffusion of Oxygen and Carbon Dioxide Through Teflon Membranes, A. M. A. Arch. Surg. 77: 938, 1958.
20. Rosen, B., and Singleton, J. H.: Automatic Recording, Gas Polymer Film Permeater, J. Polymer Sc. 25: 225, 1957.
21. Schuder, J. C., McMichael, H., McCaughan, J. S., and Kirby, C. K.: Simplified Monitor and Control Devices for Artificial Heart-Lung Machines, Tr. Am. Soc. Artificial Int. Organs, 1958.
22. Shuman, A. C.: Apparatus for Measuring the Gas Permeability of Film Materials of Low Permeability, Indust. Eng. Chem., Anal. Ed. 16: 58, 1944.
23. Simril, V. L., and Hershberger, A.: Permeability of Polymer Films to Organic Vapors, Modern Plastics 27: 95, 1950.
24. Stannett, V., and Szwarc, M.: The Permeability of Polymer Films to Gases—A Simple Relationship, J. Polymer Sc. 16: 89, 1955.
25. Waack, R., Alex, N. H., Frisch, H. L., Stannett, V., and Szwarc, M.: Permeability of Polymer Films to Gases and Vapors, Indust. Eng. Chem. 47: 2524, 1955.

(For Discussion, see page 631)

TEMPERATURE COMPENSATED, SELF-CALIBRATED OXYGEN MONITORING DEVICE*

Jesse H. Meredith, M.D. (by invitation), John H. Artesani, B.S. (by invitation), and Joseph H. Mamlin, B.S. (by invitation), Winston-Salem, N. C.

THE OXYGEN CONTENT of blood during procedures involving extracorporeal circulation has presented many new problems and challenges in medical instrumentation. One of the problems is the continuous monitoring of oxygen tension (in both venous and arterial blood) during extracorporeal circulation. One method of oxygen determination is polarography. The polarographic method of chemical analysis, invented by Jaroslav Heyrovsky,[1] is based on the unique characteristics of the current-voltage curves obtained when solutions of electro-oxidizable or electro-reducible substances are electrolyzed in a cell in which one electrode consists of mercury falling dropwise from a capillary glass tube. From such current-voltage curves it is possible not only to identify but also simultaneously determine the concentration of several or all the reducible or oxidizable substances present, i.e., simultaneously obtain a qualitative and quantitative analysis of a solution. One branch of polarography involves the use of the amperometric method. This method is used to indicate concentration measurements based on the measurement of electrolysis current at a constant potential of the indicator electrode. With this method the electrolysis current is directly proportional to the concentration of the elements being measured. For the determination of oxygen concentration, a constant potential of 0.6 volts is maintained across the electrode. The electrode consists of a platinum cathode and a silver-silver chloride anode immersed in electrolyte. The electrolyte is a saturated solution of potassium chloride. The use of this method was limited to in vitro sampling until the adaptation by Clarke and associates[2] of a gas permeable membrane to the electrode. As the result, the continuous determination of blood oxygen tension in vivo became a reality. However, because of concentration-temperature relationships (Fig. 1) it was necessary to perform determinations in a solution maintained at a constant temperature to avoid thermal

From The Department of Surgery, Bowman Gray School of Medicine, Winston-Salem, N. C.

Supported in part by grants from E. R. Squibb & Sons Co., College Gifts Program of the Western Electric Co., Inc., and gifts from the estate of J. Webb Carter.

Developed with the assistance of James M. Davis, B.S., Western Electric Co., Inc.

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

*Available from International Medical Instrument Co., Wakefield, Mass.

distortion. This paper describes an instrument which permits the continuous monitoring of oxygen tension by the polarographic (amperometric) method with automatic compensation for the temperature characteristics of the oxygen electrode. In addition, a method for introducing the polarographic electrode into the blood under sterile conditions is described.

PHYSICAL DESCRIPTION

The monitoring system is divided into three parts, (1) the oxygen electrode, (2) the cuvette for the introduction of the electrode and temperature detection of blood, and (3) electronic circuitry and read-out which allows both direct read-out and recorder output. During the original development of this instrument, the Clarke[2] electrode was used. This electrode was used during the original clinical perfusions also. We are, however, developing an experimental oxygen electrode which is an integral part of the monitoring cuvette. The cuvette* is a plastic (Teflon) cube. A stainless steel tube is introduced through

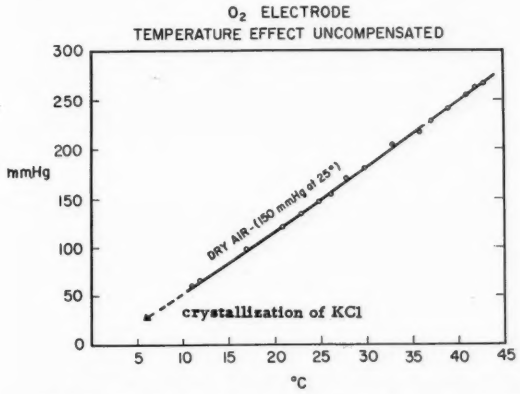


Fig. 1.—Graph showing oxygen tension versus temperature.

the Teflon cube. Perpendicular to the stainless steel tube is a chamber, at one end of which is an opening tangent to the stainless steel tube (Fig. 2). The electrode is inserted into the chamber. The surface of the electrode membrane produces a tangent to the contour of the stainless tube. Also perpendicular to the stainless steel tube and parallel with the electrode chamber is a hole into which is inserted a bead-type thermistor to make physical contact with the stainless steel tube. For clinical applications the cuvette, thermistor, electrode membrane, "O" ring, and electrolyte are autoclaved. The electrode is soaked in a solution of detergicide. The electrode is then assembled and introduced into the cuvette under sterile conditions. The cuvette is then placed in series with the arterial and/or venous line (Fig. 3). The description and evaluation of an autoclavable electrode will be described in a subsequent paper.

*Designed by Leon L. Middleman, Winston-Salem, N. C.

The electronic read-out portions consist of an entirely portable, battery operated, and transistorized unit (Fig. 4). The amplifier portion is a transistorized differential DC amplifier. The direct read-out portion consists of a 5-inch meter (Fig. 5) which is calibrated in units of millimeters of mercury on four scales which are as follows: 0-75, 0-150, 0-300, 0-750. The recorder

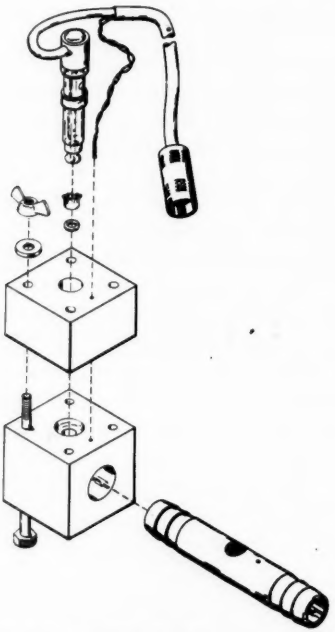


Fig. 2.—Cuvette isometric.

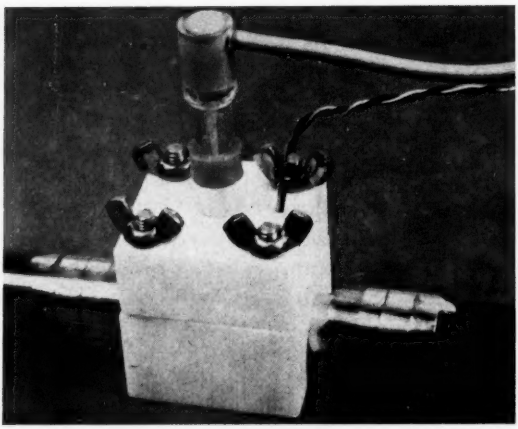


Fig. 3.—The assembled cuvette.

output portion consists of a single transistor amplifier capable of delivering sufficient output to drive most conventional recorders.

The depolarizing oxygen potential of 0.6 volts is supplied to the electrode from a battery circuit and the potential maintained within the depolarizing potential of oxygen by means of zener diode voltage regulator

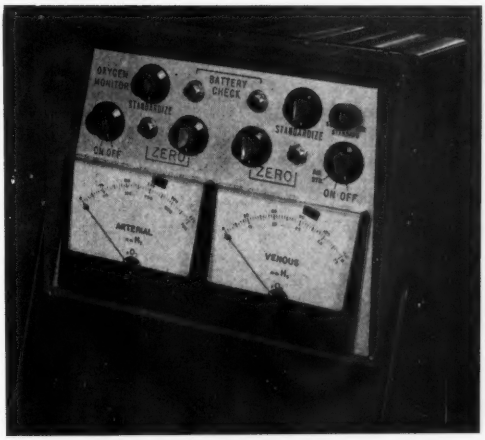


Fig. 4.—Photograph of unit.

Fig. 5.—Photograph of meter face.

Temperature compensation is achieved by the inclusion of a thermistor (Fig. 6), which is physically located in the cuvette. The requirements for temperature compensation via thermistor are that the ratio of resistance of the thermistor be as great as or greater than the ratio of the change of current output of the electrode due to temperature. The differential DC amplifier configuration assures stable operation even in the presence of atmospheric changes. The unit is standardized by exposing the electrode to room

air. Under these conditions the standardized control is moved until the partial pressure of oxygen present in room air is indicated on whichever of the four scales is desired. This method of standardization allows stable operation of the electronic circuitry regardless of the full scale range selected. The use of a transistorized circuit has resulted in several advantages: (1) the adaptability of a power supply which allows the unit to become completely portable and eliminates the necessity of power connection, (2) the physical size of the transistor circuitry allows the unit to be compact and easily portable, (3) the exceptionally low power requirements of transistor circuitry permits extended battery life. The device will operate for approximately 1,000 hours without battery replacement. In addition, the operation of a push-button type switch allows

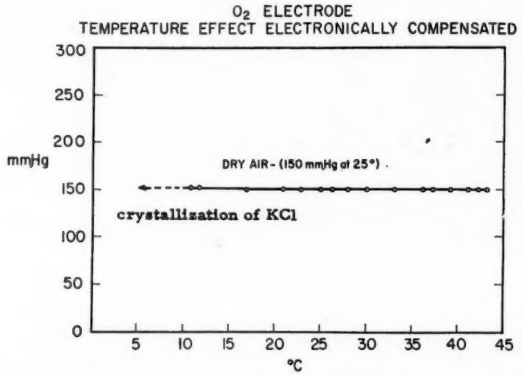


Fig. 6.—Graph showing oxygen tension versus temperature with electronic compensation.

the periodic checking of the batteries. Although this system was designed and developed for use during total body perfusion and this paper will be confined to that application, several other applications of the device have been noted and are being currently appraised. They are: (1) monitoring of O_2 during regional perfusion for cancer chemotherapy, (2) the location of shunts during cardiac catheterization, and (3) as part of an experimental instrumentation system for the determination of O_2 consumption of various organs in vivo. For purposes of total body perfusion, a double unit is used; one continuously indicating the oxygen tension of arterial blood and the second unit continuously indicating the oxygen tension of venous blood. The electrodes are standardized in room air under sterile conditions prior to their insertion into the cuvette. They are checked in blood during a recirculation period. During this time both the venous and arterial oxygen tension will be identical. This reading during recirculation allows the adequate pre-perfusion oxygenation of the priming blood. During the initial use of this instrument during perfusion, it was noted that the oxygen tension of donor blood remained relatively low for a long period of time even during recirculation. This period of low oxygen tension during recirculation and oxygenation is a result of oxygen uptake by the hemoglobin with a minimal

elevation of the partial pressure of oxygen. By periodic sampling or the use of a continuous pH monitor, it was demonstrated that during the period of recirculation the pH of the priming blood increased. As a result of these observations the degree of oxygenation of priming blood and the control of pH was maintained and brought to normal point prior to the time of going on bypass. Close contact is maintained with the surgeon upon going on full bypass. The venous oxygen monitor is watched for the first bolus of patient blood passing through the cuvette. This bolus of blood will give an indication of the patient's venous oxygen tension. During normothermia this level is readily maintained; however, during hypothermia and severe hypothermia, a decrease in venous oxygen tension has been noted. This decrease in plasma oxygen tension is reasonable when one considers the increased combining power of hemoglobin at a lowered temperature.

The oxygen monitor has been used during clinical perfusions and laboratory experiments. It has been found to be a valuable instrument which allows continuous monitoring of this vital parameter.

SUMMARY

This paper describes an instrument which permits the continuous monitoring of oxygen tension in vivo during normothermia and severe hypothermia. A method for introducing the detecting device into blood under sterile conditions and the electronic circuitry are described.

REFERENCES

1. Jaroslav Heyrovsky: Chem. Listy **16**: 256, 1922.
2. Clarke, L. C., Wold, R., Granger, R., and Taylor, Z.: J. Appl. Physiol. **6**: 189, 1953.

(For Discussion, see page 631)

PULMONARY ARTERIAL HYPEROXIA PRODUCING INCREASED PULMONARY VASCULAR RESISTANCE

Peter V. Moulder, M.D., Joseph R. Lancaster, M.D. (by invitation), Robert W. Harrison, M.D. (by invitation), Stephen L. Michel, B.S. (by invitation), Mark Snyder, M.D. (by invitation), and Richard G. Thompson, M.D. (by invitation), Chicago, Ill.

THE RELATIONSHIP of oxygen and lack of oxygen to the pulmonary circulation has been intensively studied in the laboratory and the relationship has been further used in cardiac catheterization studies, as well as being applied to clinical medicine. In 1946 von Euler and Liljestrand[1] first demonstrated the effect of hypoxia on the pulmonary circulation with the induction of pulmonary hypertension. This was further demonstrated in normal human beings and in patients with disease of the pulmonary vascular circulation in 1947 by Motley and Cournand,[2] and by many others[3, 4, 5] in subsequent years. There has not been a clear-cut refutation of this effect of hypoxia in human studies. Helen Duke[6] has demonstrated this same hypoxic effect in elaborate perfusion and cross-circulation experiments in cats. Others have found this reaction in cats, rabbits, and dogs.[7-10] Although there are still repeated observations on hypoxia increasing pulmonary vascular resistance, there have appeared recently a few studies[11-14] which show no such effect. The most critical and neatest set of observations were those of Nahas and co-workers[11] in unanesthetized dogs trained for study in whom the effect of hypoxia in chamber breathing revealed no increased pulmonary vascular resistance. This study emphasized the fact that the Fick method of measuring cardiac output was unreliable in these unsteady states of hypoxia and that the dye curve method of Stewart and Hamilton was the most useful system. Nahas demonstrated that, although the pulmonary pressure became elevated, it was merely a reflection of an increased cardiac output and that there was little change in the vascular resistance in the lungs.

There has been little or no experimental work on the effect of oxygen at the pulmonary arterial level. There have been sporadic studies related to inspired oxygen concentration changes and to systemic-pulmonary shunts. Breathing 100 per cent oxygen, as well as the injection of certain drugs, such as acetylcholine and aminophylline, has been used to demonstrate lability of the pulmonary vascular bed in the patient with pulmonary hypertension. Ferguson and Varco[15, 16] and Muller and Dammann[17] have studied the effects of systemic

From the Department of Surgery of The University of Chicago, Chicago, Ill.
Supported by a grant from the John A. Hartford Foundation.
Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

to pulmonary arterial shunts on the pulmonary circulation, and, in general, the attempt to produce pulmonary hypertension without the separation of a lobe from the pulmonary circulation has been disappointing. Studies of large left-to-right shunts from the systemic circulation to the right atrium in this laboratory[18] have shown minimum effect on the pulmonary vascular resistance. In the studies in dogs by Boake, Daley, and McMillan,[19] in 1959, studying the effect of a partial reoxygenated circulation (extracorporeally produced) in reversing hypoxic-induced pulmonary hypertension, a slight increase in pulmonary arterial pressure was noted when the extracorporeal circuit was added to the normal circulation.

These experiments were planned to approach complete control of the blood and atmospheric oxygen concentrations contacting the pulmonary vascular bed so that the inter-relationship of these two could be studied. The findings of a tremendous increase in pulmonary vascular resistance with the introduction of oxygenated blood to the pulmonary circulation has overshadowed the study of the relationships of inspired gas to blood-gas concentration.

METHOD

Healthy mongrel dogs, weighing from 18 to 22 kilograms, received 2 mg. per kilogram of morphine sulfate subcutaneously followed by Thiobarbital (2.5 per cent) administered intravenously until light anesthesia was obtained. Open tracheostomy with a large, securely-fitting metal cannula allowed complete control of the gas mixtures used for ventilation. The gas was delivered from tanks containing various percentages of nitrogen and oxygen and an intervening Rattenborg[20] valve, which allowed for dissipation of the expired gas and continuous replacement of the inspired gas with the planned concentration from the gas cylinder. With the thorax open, ventilation was performed manually at the rate of 18 per minute with sufficient volume to bring the lungs to the chest wall. Right thoracotomy through the fifth intercostal space was performed and cannulations with large bore catheters were made as follows: through the azygos vein—right atrium and right ventricle; through a small branch of the pulmonary vein—left atrium; and through a branch of the pulmonary artery—central pulmonary artery, and a separate catheter placed with this was used for withdrawing pulmonary arterial blood samples for analyses. Statham P23G, P23D, or P23DC pressure transducers were used and all records were taken on a six-channel Grass polygraph with Lead II electrocardiogram.

Indocyanine green dye dilution curves were performed for estimation of the cardiac output. These were recorded on a Texas direct writing rectilinear galvanometer at a paper speed of 12 in. per minute. The dye was injected rapidly into the right ventricle and blood was withdrawn from the thoracic aorta at a rate of 80 ml. per minute. Gilford withdrawal syringe and densitometer were used. Sodium heparin was given intravenously at a dose of 2 mg. per kilogram just before instituting extracorporeal circulation. Cannulations were performed as follows: the superior vena cava was drained

via a large catheter passed through the external jugular vein to a point above the azygos vein in the superior vena cava with total occlusion by tape tie obtained when bypass was instituted; a large bore catheter was placed in the inferior vena cava after it was ligated and opened to assure total bypass; venous blood was drained by gravity to a well; shunted blood was returned from an overflow well system at a constant level above the right atrium via a large catheter in the right atrial appendage, and the overflow from this double funnel system drained to a well parallel with the caval drainage one. These were then pumped by means of a Sigmamotor TM4 pump through a Brown-Harrison heat exchanger and Abbott stainless steel mesh filter to the

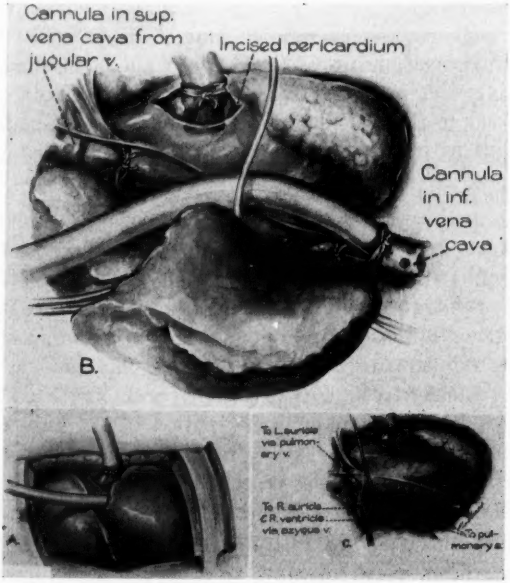

Cannula in sup. vena cava from jugular v.
Incised pericardium
Cannula in inf. vena cava
B.
To L auricle via pulmonary v.
To R auricle & R ventricle via azygous v.
C.
To pulmonary a.

Fig. 1.—Position of blood catheters and measuring cannulae. *A*, During study period catheter positions are such as not to interfere with motion of lung. *B*, The caval cannulae positioned to ensure complete venous drainage. *C*, The sites of the various catheters for pressure measurements and other studies.

constant level reservoir. For oxygenation of the venous blood, the blood from the two wells was shunted through a double set of bubble oxygenators, Pulmo-Paks,* and thence returned to the venous overflow reservoir for subsequent return to the right atrium. Fig. 1 demonstrates the relationship of these various catheters in the chest and in Fig. 2 the extracorporeal setup for returning venous blood or oxygenated blood. The pump speed is fixed to produce constant overflow at all times and the system primed with fresh, compatible, heparinized dog blood. Temperature control was maintained with the use of the heat exchanger in the circuit and temperatures were monitored with a Yellow Springs telethermometer by thermistor probes in the constant level reservoir, rectum, and in deep neck muscle.

*Pulmo-Pak bubble oxygenators were generously supplied by Dr. Rodney Gwinn of Abbott Laboratories.

After cannulations were completed except for the one in the inferior vena cava, the control readings were obtained. After cannulation of the inferior vena cava and the initiation of simple bypass, the level of the constant reservoir was adjusted to reproduce nearly right atrial and systemic pressure without elevation of right ventricular diastolic pressure. This was surprisingly constant at a level of 20 cm. above the table and about 10 cm. above the superior vena cava-right atrial junction. Electrolyte and acid-base chemical analyses and microhematocrit determinations were performed on thoracic aortic blood by standard methods[21]; blood oxygen saturation and CO_2 content were determined by Van Slyke procedures[22] on thoracic aortic blood, pulmonary arterial and venous well blood to represent mixed venous sample when bypass oxygenation was instituted.

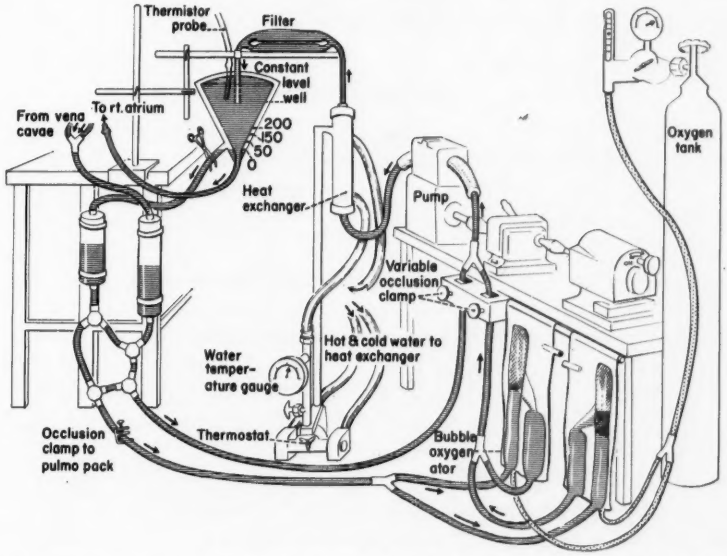


Fig. 2.—Diagrammatic presentation of the various elements in the extracorporeal circuit which allowed return of venous blood or oxygenated blood from a constant level overflow reservoir with Brown-Harrison heat exchanger for temperature control.

RESULTS

Eleven useful experimental preparations were obtained (if the procedure had been excessively traumatic or pressure pulse curves were not adequate for study, or if there had been a period of failure or difficulty with the heart, the study was not continued). Ten of these animals were prepared as just described with an irreversible bypass in which certainty of total venous circulatory bypass was desired (exclusive of coronary venous blood). However, one animal was prepared for reversible bypass in which the inferior caval cannulation was performed through the right atrial wall.

Of the 10 dogs with irreversible bypass, there were slight variations in pattern. First, the initial searching studies were primarily aimed toward studying the relations of expired blood gas and, as the hyperoxygenation effect was

noted, the pattern of study was modified accordingly. Measurement of pulmonary wedge pressure, in order to see an effect on the pulmonary veins between pulmonary wedge and left atrium, was attempted, but good curves were obtained so infrequently that these data are useless. Use of overflow blood as an indicator of cardiac output proved to be a surprisingly misleading method: the pump was set at a fixed output, the amount of blood from a timed collection in the over-

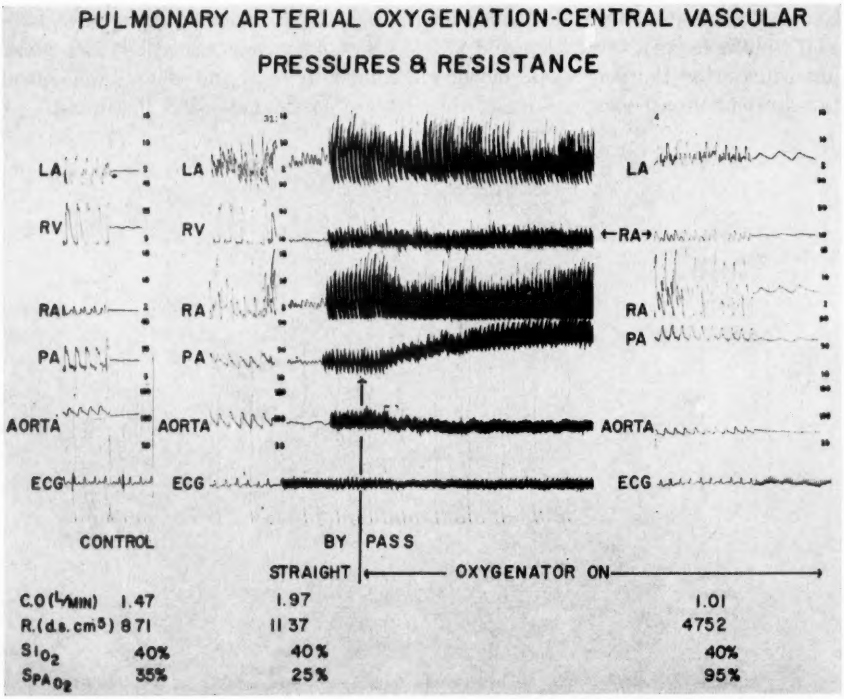


Fig. 3.—Reproduction of segments of the original pressure pulse traces with oxygen values and calculated cardiac output and pulmonary vascular resistances obtained on a dog developing a lethal increase in pulmonary vascular resistance under the stimulus of oxygenated blood. The rise in pulmonary arterial pressure is seen in the central panel with recording at slow speed.

flow system subtracted from the predetermined output of the pump, and flow from the reservoir to right atrium was calculated; however, this proved to be unrelated to dye dilution curve studies. Second, some of these animals during the run actually died from the induced pulmonary hypertension. This is graphically demonstrated in Fig. 3, in which an animal with abnormally high initial resistance of 871 dyne sec. cm.$^{-5}$, associated with a pulmonary arterial oxygen saturation of 35 per cent, shows an increasing resistance to 1,137 on bypass and, with oxygenation, a striking increase in pulmonary artery pressure demonstrated on the slow speed record of pressures. After a short time on the bypass with oxygenator, there is a decrease in cardiac output with a tremendous increase in vascular resistance to 4,752 dyne sec. cm.$^{-5}$ Shortly thereafter, the

right heart failed and death ensued. Third, the use of 8 per cent oxygen in the inspired gas concentration produced a pattern of such rapid deterioration that an adequate study could not be performed; therefore, in subsequent experiments, stabilization at higher oxygen concentration was performed first.

Three experiments were performed in almost precisely the same pattern. In these three, there were nine separate stages of study: (1) control run without bypass with the animal breathing 40 per cent oxygen; (2) simple bypass, breathing 40 per cent oxygen; (3) bypass with oxygenator, breathing 40 per cent oxygen; (4) bypass with oxygenator, breathing 8 per cent oxygen; (5) bypass with oxygenator, breathing 100 per cent nitrogen, no oxygen; (6) bypass with oxygenator, breathing 20 per cent oxygen; (7) simple bypass, breathing 20 per cent oxygen; (8) bypass with oxygenator, breathing 8 per cent oxygen; and (9) simple bypass, breathing 8 per cent oxygen. Three readings were made in the ninth stage before termination of the experiment and, because of the low oxygenation, usually pre-terminus in the animal. Table I lists these data obtained on the 3 animals carried through this experiment.

One experiment was performed as described with ability to come off the oxygenator and bypass completely, and this served to corroborate the findings with the irreversible bypass.

In general, on the circulatory bypass, with or without oxygen, there was some increase in pulmonary arterial pressure and pulmonary vascular resistance. This is demonstrated in Fig. 4, in which the various stages, as just described, on the 3 strictly comparative animals are presented, with variations from the control measurement base line averaged and graphed in percentage deviations. At all times on the bypass, the 3 animals showed increased pulmonary artery minus left atrial mean pressure and increases in total pulmonary resistance with minor variations in cardiac output. However, the presence of the oxygenating shunt was associated with the most marked increases in vascular resistance. There is a suggestion in the pattern seen in stages 3, 4, and 5, in which the respiratory gas oxygen concentration was decreased, that there might be an additive effect of the inspired low gas oxygen concentration. However, this was not necessarily true in other experiments and, since there is variation in the $S_{PA_{O_2}}$, this would be attempting to extract too much from the data with reference to this one variable.

The experimental setup was associated with a remarkably stable pulse, electrocardiogram, and central aortic pressure, which lends validity to these data. Fig. 5 presents segments of the pressure curves and electrocardiogram from one such experiment (right ventricular pressure was on another polygraph at this time when a pulmonary wedge pressure was being sought). The uselessness of the wedge curve throughout the experiment is apparent. There is an increase in phasic and mean pulmonary artery pressures when bypass is instituted, and these further increase with the oxygenator. There is a reversion with the oxygenator off, although they remain higher than those prior to institution of bypass. The importance of the recording of left atrial phasic and mean pressures can be seen in the third and fourth panels when the atrial pressure has also risen with the pulmonary artery pressure and accuracy can be obtained

TABLE

DOG NO.	CONDITION	TEMPERATURE (° C.)			BLOOD						CHEMISTRIES SERUM (mEQ./L.)			
					MIXED VENOUS		PULMONARY ARTERY		AORTA					
		RECTAL	MUSCLE	BLOOD	O_2 SAT. (%)	CO_2 (VOL. %)	O_2 SAT. (%)	CO_2 (VOL. %)	O_2 SAT. (%)	CO_2 (VOL. %)	Na	K	Cl	CO_2
	Control Run													
1	Pre-Bypass Breathing:	37.0	37.0	–	–	–	40.88	40.88	95.62	27.63	–	–	–	18.5
2	60% N_2—40% O_2	37.0	37.0	–	–	–	32.4	–	95.9	–	–	–	–	17.5
3		–	35.0	–	–	–	60.83	31.25	85.11	31.02	–	–	–	17.2
	Straight Bypass													
1	Breathing:	36.2	36.5	38.0	–	–	43.84	33.18	87.49	28.09	139	4.5	113	15.5
	60% N_2—40% O_2													
2		37.2	37.5	38.0	–	–	49.33	32.73	100.43	23.18	145	3.3	121	12.2
3		–	36.2	38.0	–	–	53.94	28.90	86.15	22.63	144	4.8	120	14.1
	Bypass Oxygenator													
1	On Breathing:	36.6	36.7	37.8	43.31	32.53	96.01	20.23	95.57	20.18	–	–	–	11.5
	60% N_2—40% O_2													
2		37.0	37.8	38.5	52.23	29.44	94.76	22.11	98.79	17.96	–	–	–	11.3
3		–	36.5	37.2	60.13	24.16	80.25	22.56	100.79	15.57	–	–	–	8.9
	Bypass Oxygenator													
1	On Breathing:	36.6	36.5	37.5	44.72	27.86	91.33	18.94	91.09	18.15	–	–	–	10.6
	92% N_2—8% O_2													
2		37.0	38.0	38.4	46.64	26.46	89.94	18.49	89.51	16.70	–	–	–	9.6
3		–	36.2	37.0	50.05	24.51	106.83	17.26	94.84	15.36	–	–	–	9.1
	Bypass Oxygenator													
1	On Breathing:	36.5	36.4	37.7	46.13	26.95	101.22	20.72	89.93	17.54	–	–	–	9.9
	100% N_2													
2		37.3	38.2	38.0	30.87	23.48	97.40	19.27	67.89	14.08	–	–	–	8.6
3		–	37.0	37.0	41.45	22.09	98.23	16.25	91.68	15.45	–	–	–	8.7
	Bypass Oxygenator													
1	On Breathing:	36.5	36.4	37.7	–	–	–	–	–	–	–	–	–	–
2	80% N_2—20% O_2	37.4	38.1	38.0	–	–	–	–	–	–	–	–	–	–
3		–	37.0	38.0	–	–	–	–	–	–	–	–	–	–
	Bypass Oxygenator													
1	Off Breathing:	36.2	36.6	38.0	–	–	53.58	24.29	63.70	22.80	141	4.5	111	12.0
2	80% N_2—20% O_2	37.0	38.0	38.2	–	–	43.24	23.68	72.93	20.30	144	3.0	116	11.7
3		–	37.0	38.0	–	–	23.10	27.82	52.18	21.33	138	4.6	120	11.6
	Bypass Oxygenator													
1	On Breathing:	–	–	–	–	–	–	–	–	–	–	–	–	–
2	92% N_2—8% O_2	37.0	38.0	37.5	–	–	–	–	–	–	–	–	–	–
3		–	–	–	–	–	–	–	–	–	–	–	–	–

(Continued on next pages.)

BLOOD						PRESSURES (MM. Hg.)							
CHEMISTRIES SERUM (MG. %)				CELL VOLUME (%)	PULSE	RIGHT ATRIUM	RIGHT VENTRICLE	PULMONARY ARTERY	LEFT ATRIUM	AORTA	MEAN PA-LA	CARDIAC OUTPUT (L./MIN.)	TOTAL PULM. RESISTANCE (DYNE SEC. CM.$^{-5}$)
pH	Ca	P	NPN										
						2/0		30/8	12/8	120/90			
7.31	–	–	–	47	135	M = 1	30/0	M = 17	M = 10	M = 100	7	1.23	455
						6/0		28/10	4/0	140/110			
7.32	–	–	–	52	170	M = 2	30/0	M = 13	M = 1	M = 120	12	2.18	440
						4/0		21/5	3/0	90/60			
7.27	–	–	–	45	152	M = 2	22/0	M = 10	M = 2	M = 70	8	1.56	410
						2.5/–1		30/19	8/5	140/105			
7.32	9.5	5.5	–	–	125	M = 1.5	30/0	M = 25	M = 7	M = 120	18	1.33	1083
						6/2		30/15	3/0	160/110			
7.28	9.5	6.0	28	52	120	M = 4	30/0	M = 20	M = 1	M = 130	19	1.98	768
						4/0		23/10	3/0	90/60			
7.21	–	–	33	45	147	M = 1.5	25/0	M = 13	M = 2	M = 70	11	1.74	506
						4.5/2.5		40/30	15/8	150/100			
7.35	–	–	–	50	130	M = 3.5	40/2	M = 34	M = 11	M = 120	23	1.13	1628
						9/5		38/20	5/0	160/110			
7.35	–	–	–	49	143	M = 8	38/2	M = 27	M = 3	M = 120	24	1.79	1073
						4/0		26/15	3/0	100/75			
7.35	–	–	–	41	139	M = 2.2	26/0	M = 18	M = 2	M = 85	16	1.70	753
						4/0		40/27.5	10/6	140/95			
7.43	–	–	–	46	170	M = 3	40/1.5	M = 30	M = 9	M = 110	21	1.20	1400
						11/7		43/27	3/0	150/100			
7.31	–	–	–	49	182	M = 8	43/3	M = 35	M = 1	M = 110	34	1.98	1374
						6/2		35/25	3/1	100/80			
7.36	–	–	–	47	140	M = 4	32/0	M = 27	M = 2	M = 85	25	1.97	1015
						4/0.5		40/32.5	11/7	145/100			
7.40	–	–	–	44	170	M = 3.5	40/4	M = 35	M = 10	M = 110	25	1.05	1905
						5/2		35/20	3/0	115/80			
7.36	–	–	–	52	160	M = 4	37/0	M = 25	M = 1.5	M = 100	23.5	2.09	900
						4/0		35/27	3/0.5	85/65			
7.39	–	–	–	45	170	M = 2	35/0	M = 31	M = 2	M = 70	29	1.68	1381
						3.5/0.5		45/32.5	10/4	135/100			
–	–	–	–	–	170	M = 3.0	45/5	M = 35	M = 8.5	M = 110	26.5	0.93	2279
						7/2		35/20	3/0	105/75			
–	–	–	–	–	200	M = 5	N.G.	M = 25	M = 1	M = 90	24	1.88	1021
						6/2		42/26	4.5/1	95/60			
–	–	–	–	–	148	M = 4	36/0	M = 32	M = 2.5	M = 80	29.5	1.91	1236
						8/5		40/32.5	12/8	160/130			
7.38	9.5	6.6	–	44	170	M = 6.5	40/5	M = 35	M = 10	M = 140	25	1.34	1493
						6/3		37/20	6/3	120/90			
7.23	9.7	6.7	33	52	120	M = 5	37/0	M = 23	M = 4	M = 100	19	1.96	775
						2.5/0		30/20	2/0	80/55			
7.26	–	–	42	45	170	M = 1.5	30/0	M = 25	M = 1	M = 60	24	1.97	975
						6/4		40/32.5	11/8	140/110			
–	–	–	–	–	150	M = 5.5	40/2.5	M = 35	M=10.5	M = 115	24.5	1.15	1704
						6/2		28/17	2/0	70/40			
–	–	–	–	–	180	M = 4	N.G.	M = 22	M = 1	M = 60	21	1.76	955
						4/0		37/27	2/0	80/60			
–	–	–	–	–	177	M = 2	33/0	M = 31	M = 1	M = 70	30	1.52	1579

TABLE

DOG NO.	CONDITION	TEMPERATURE (° C.) RECTAL	MUSCLE	BLOOD	BLOOD: MIXED VENOUS O_2 SAT. (%)	MIXED VENOUS CO_2 (VOL. %)	PULMONARY ARTERY O_2 SAT. (%)	PULMONARY ARTERY CO_2 (VOL. %)	AORTA O_2 SAT. (%)	AORTA CO_2 (VOL. %)	CHEMISTRIES SERUM (mEq./L.) Na	K	Cl	CO_2
	Bypass Oxygenator													
1	**Off Breathing:**	36.1	36.4	38.0	–	–	55.33	25.70	55.43	24.59	–	–	–	–
2	92% N_2—8% O_2	37.4	38.0	38.2	–	–	30.33	23.44	46.40	17.27	–	–	–	11.1
3		–	37.0	37.2	–	–	28.96	22.35	44.50	18.94	–	–	–	9.8
	Bypass Oxygenator													
1	**Off Breathing:**	35.9	35.6	38.0	–	–	–	–	12.65	27.50	–	–	–	–
2	92% N_2—8% O_2	–	–	–	–	–	9.54	24.61	26.15	19.10	–	–	–	–
3	"	–	37.0	37.2	–	–	9.73	24.26	15.77	15.33	–	–	–	–
	Bypass Oxygenator													
1	**Off Breathing:**	–	–	–	–	–	–	–	–	–	–	–	–	–
2	92% N_2—8% O_2	37.0	38.0	38.0	–	–	–	–	–	–	–	–	–	–

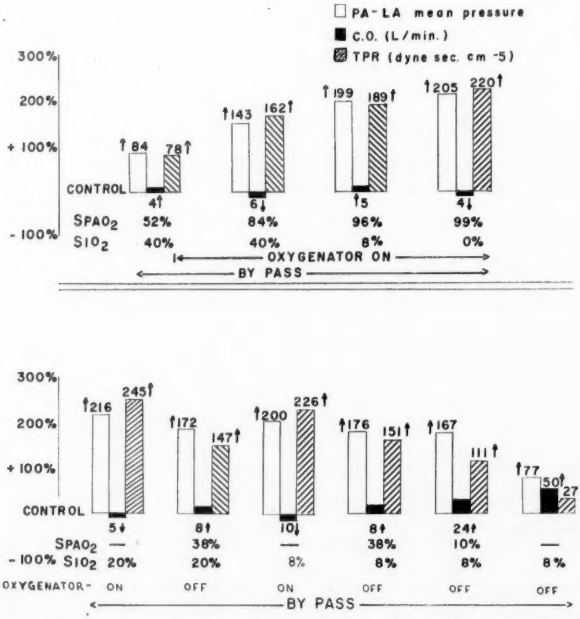


Fig. 4.—Comparison of the important circulatory findings in the nine stages of study on three experiments from the data in Table I. Each value is compared to its control level and the average percentage change for the three experiments is represented in the blocks for effective mean pulmonary artery pressure (PA-LA mean), cardiac output, and total pulmonary resistance.

I—CONT'D

BLOOD						PRESSURES (MM. Hg.)								
CHEMISTRIES SERUM (MG. %)				CELL VOLUME	PULSE	RIGHT ATRIUM	RIGHT VENTRICLE	PULMONARY ARTERY	LEFT ATRIUM	AORTA	MEAN PA-LA	CARDIAC OUTPUT (L./MIN.)	TOTAL PULM. RESISTANCE (DYNE SEC. CM.$^{-5}$)
pH	Ca	P	NPN	(%)									
						5/3		40/32.5	13/10	165/135			
–	–	–	–	47	150	M = 4.0	40/1	M = 35	M = 12	M = 150	23	1.51	1219
						12/4		30/20	5/2	138/100			
7.26	–	–	–	49	140	M = 6	30/0	M = 23	M = 3	M = 120	20	1.92	833
						4/0		37/25	2.5/0	100/70			
7.27	–	–	–	48	170	M = 2	N.G.	M=27.5	M = 1	M = 80	26.5	1.74	1218
						14/5		40/25	20/10	200/130			
–	–	–	–	–	50	M=N.G.	40/4	M = 35	M = 14	M = 160	21	1.61	1043
						6/2		30/15	3/0	120/90			
–	–	–	–	–	130	M = 5	N.G.	M = 22	M = 1	M = 105	21	2.25	747
						4/0		40/25	3/0	100/55			
–	–	–	–	–	178	M =2.5	N.G.	M = 28	M = 2	M = 70	26	2.17	958
						12/2		20/10	5/2	120/40			
–	–	–	–	–	80	M = 4	20/2	M = 15	M = 2.5	M = 60	12.5	2.35	426
						7/2		33/15	3/0	100/65			
–	–	–	–	–	110	M = 5	N.G.	M = 22	M = 1	M = 90	21	2.40	700

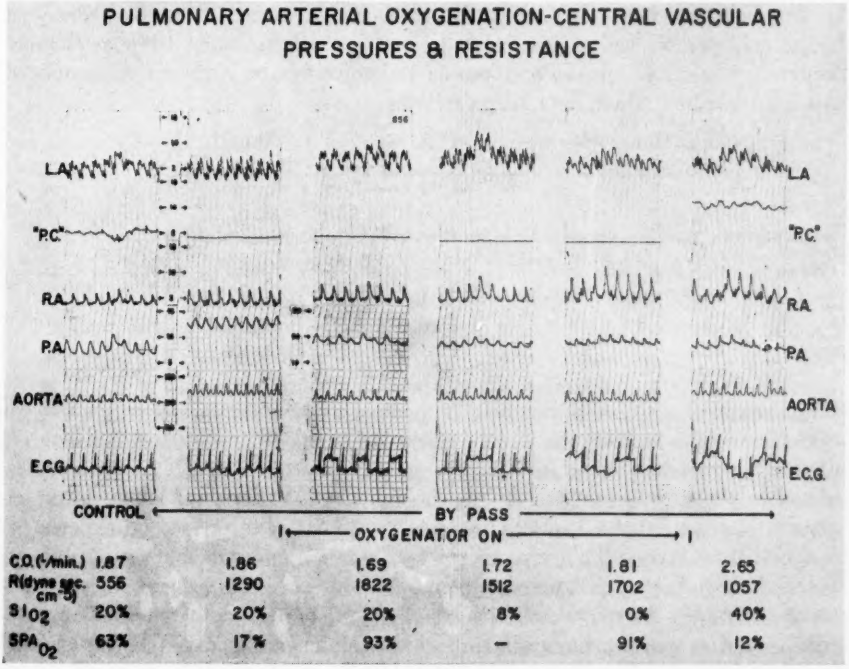


Fig. 5.—Segments of original pressure pulse traces with the oxygen data and calculated cardiac output and total pulmonary resistance for one experiment demonstrating characteristic changes associated with bypass, and bypass with oxygenation, and, under these circumstances, no added effect from decrease in inspiratory oxygen concentration (SIo_2).

only when the pulmonary artery minus left atrial mean pressure is used for calculating the total pulmonary resistance. There is steady control of output, and the usefulness of the constant level well is seen. Data on the 3 animals carried through the nine-stage experiment, as seen in Table I and Fig. 4, show these same phenomena. Examples of the lack of correlation between decrease in inspired oxygen concentration on the resistance change in these circumstances can also be seen.

With such a complex experiment, a number of monitors and controls were instituted to keep as stable a preparation as possible. If a heat exchanger were not in the system, the temperature fell to 29° to 31° C.; therefore, the heat exchanger in the circuit is mandatory. Temperature measurements at the blood, muscle, and rectal sites indicated stability at 36° to 38° C. in all experiments. At least two electrolyte checks were obtained and little change found. Repeated acid-base studies indicated no profound abnormality—a mild, metabolic acidosis was occasionally present, as is often associated with surgical procedures and anesthesia, and a respiratory alkalosis was usually present, often compensating the acidosis, reflecting not only hyperventilation but decarboxylation by the bubble oxygenator. Whole blood hematocrit measurements changed little throughout the procedure. There were occasional instances of pulse abnormality. In 1 animal not used for this study, there was a period of bradycardia just before going on bypass, and calculated resistance did not vary, whereas changes occurred in cardiac output and phasic pulmonary artery pressure because of this bradycardia. These data are as follows:

Pulse—48/min.	P.A. = 27/5 $\overline{12}$ mm. Hg
	$PA_m - LA_m$ = 10 mm. Hg
	C.O. = 1.96 L./min.
	2.19 L./min.
	R = 408 dyne sec. cm.$^{-5}$
	365 dyne sec. cm.$^{-5}$
Pulse—78/min.	P.A. = 22/15 $\overline{17}$ mm. Hg
	$PA_m - LA_m$ = 13 mm. Hg
	C.O. = 2.47 L./min.
	R = 421 dyne sec. cm.$^{-5}$

Right ventricular pressure was used to monitor ventricular diastolic pressure for evidence of failure; however, when a marked change occurred, the cannula pulled back into the right atrium (see Fig. 3) and, as noted in Table I, an occasionally unusable pressure curve was obtained. In rare instances, an acute, but temporary, elevation of the diastolic pressure was found as in the second experiment of Table I. During the times of marked increase in pulmonary vascular resistance, there is a modest increase in the diastolic pressure reflected in the right atrial pressure. Furthermore, the systolic level of the right ventricular pressure served as a check on the systolic level in the pulmonary artery, the most important pressure parameter.

A portion of lung was biopsied from each side at the end of the procedure to check for thromboses, arterial abnormalities, worms, etc. The heart and major proximal vessels were opened and examined grossly for the same reasons. None was noted.

DISCUSSION

The oxygenation of the pulmonary artery blood under these circumstances is 30 to 60 per cent above that of the usual mixed venous oxygen saturation, and from a pulmonary circulatory point of view, it is therefore hyperoxic. Smith,[23] Bennett[24] and their associates, in 1932-1934, demonstrated pathologic pulmonary arterial changes and elevation of pulmonary artery pressure in rats when the animals were subjected to chronic elevation of atmospheric pressure, and it is notable that the environmental oxygen tension was 635 mm. Hg. They felt that the pulmonary hypertension may have been present early but had not been measured. It is clear that one of the next steps in this study is to control the oxygenation and to measure Po_2, as well as the oxygen saturation, especially when saturations of 100 per cent oxygen are obtained.

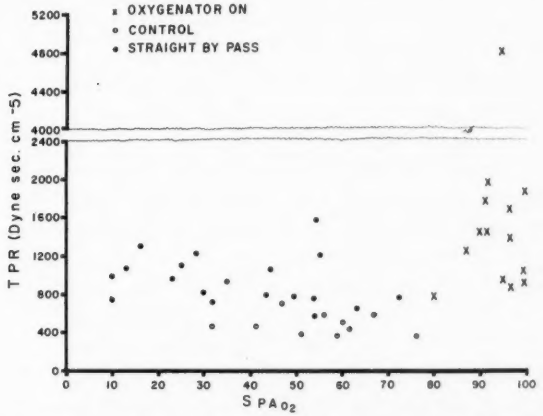


Fig. 6.—Plot of values of pulmonary arterial oxygen saturation ($SPAo_2$) and calculated total pulmonary resistance (TPR) (when obtained simultaneously) under the three experimental circumstances (control, bypass, and oxygenator) in an attempt to elucidate the effect of hypoxia, as well as hyperoxia, on vascular resistance.

How does this hyperoxic reaction relate to shunts? At the present time an extensive survey is being done on our own and published catheterization data to see if there is any relationship between the measured pulmonary arterial oxygen saturation and the measured pulmonary vascular resistance; however, it would seem that there is not such a correlation. For example, the large interatrial septal defects are commonly associated with a high value of mixed venous oxygen saturation and, in general, are usually associated with normal or low pulmonary vascular resistances. On the other hand, small shunts from the high pressure circuit in ventricular septal defect and patent ductus can be associated with increased vascular resistance. The direct pressure effect, jet effect, laminar flow of hyperoxygenated blood (otherwise well mixed from an atrial side), striking pulmonary receptors from a high pressure source, or some other factor may be operating.

In many instances the presence of the bypass itself, without the bubble oxygenator in the system, was associated with increased pulmonary vascular resistance. However, two things must be noted. The bypass is initiated with an

oxygenated blood drawn from the donors' arterial systems and, in two instances, measurement of the saturation has shown it to be high. For example, in the dog with the reversible bypass, the initial institution of bypass was associated with a donor blood oxygen concentration of 91 per cent and there was a striking increase in pulmonary arterial pressure, cardiac output, and resistance. As time progressed and the saturation fell to 67 per cent, there was a return toward normal, but not to normal, of these circulatory dynamics. Reverse of the bypass to control produced a normal pattern; then reinstitution of the bypass from a circuit now containing venous oxygenated blood of close to 60 per cent produced little or no change in resistance and only a slight increase in output and pressure. Addition of the oxygenator, inducing a pulmonary artery blood oxygen concentration of 100 per cent, produced a marked increase in pulmonary vascular resistance. Bypass alone produced reversion of vascular resistance close to normal. Return to normal circulation reproduced control resistances, although associated with a somewhat lower cardiac output.

Could there be a bifid reaction; that is, a modest stimulus to increased vascular resistance from decreased oxygen concentration and a marked stimulus with increased oxygen concentration? Fig. 6 shows a plot of data in which a pulmonary arterial oxygen saturation was obtained at the same time as total pulmonary resistance was calculated and, as can be seen with the three groups of studies—control, straight bypass, and bypass with oxygenator—there is a tendency to a somewhat increased resistance at the lower oxygen concentration and a clear-cut indication of increased resistance at high oxygen concentrations. There is too much scatter and too few points to judge the hypoxic effect, especially in view of the variation in circumstances of measurement.

There are certain clinically useful suggestions from this study. In the pulmonary hypertensive patients undergoing extracorporeal circulation in whom the lungs fill with blood from the systemic side of the circuit and are usually ventilated with gas of high oxygen concentration, the stage for a further increased pulmonary vascular resistance, obtainable at the time of coming off of bypass, might be set, and some variation in the inhaled gas mixture might be worthy of study. The possible deleterious effect of small shunts, and especially small residual post-surgical shunts in patients with pulmonary hypertension, may assume great significance.

The increased pulmonary vascular resistance under hypothermia might also be explained by this mechanism, as the oxygen saturation of mixed venous blood increases as a result of decreased peripheral metabolism and arteriovenous shunting.

SUMMARY

Observations with the use of a controlled extracorporeal shunt from venae cavae to right atrium in which the oxygenation of the blood, other than that returning by the coronary sinus, can be controlled, have shown an increased vascular resistance associated with the institution of the bypass circuits and, most importantly, a striking increase in all parameters related to increased total

pulmonary resistance and pulmonary hypertension when excessively oxygenated blood is returned to the right side of the heart. The vascular reaction to oxygenated blood in the pulmonary circuit has even been lethal in normal dogs.

The technical assistance of Florence Kosmal and William Rank is gratefully acknowledged.

REFERENCES

1. Von Euler, U. S., and Liljestrand, G.: Observations on the Pulmonary Arterial Blood Pressure in the Cat, Acta physiol. scandinav. **12**: 301, 1946.
2. Motley, H. L., Cournand, A., Werko, L., Himmelstein, A., and Dresdale, D.: The Influence of Short Periods of Induced Acute Anoxia Upon Pulmonary Artery Pressures in Man, Am. J. Physiol. **150**: 315, 1947.
3. Westcott, R. N., Fowler, N. O., Scott, R. C., Harrenstein, V. D., and McGuire, J.: Anoxia and Human Pulmonary Vascular Resistance, J. Clin. Invest. **30**: 957, 1951.
4. Fishman, A. P., McClement, J., Himmelstein, A., and Cournand, A.: Effects of Acute Anoxia on the Circulation and Respiration in Patients With Chronic Pulmonary Disease Studied During the "Steady State," J. Clin. Invest. **31**: 770, 1952.
5. Doyle, J. T., Wilson, J. S., and Warren, J. V.: The Pulmonary Vascular Responses to Short Term Hypoxia in Human Subjects, Circulation **5**: 263, 1952.
6. Duke, H. N.: The Site of Action of Anoxia on the Pulmonary Blood Vessels of the Cat, J. Physiol. **125**: 373, 1954.
7. Aviado, D. M., Ling, J. S., and Schmidt, C. F.: Effects of Anoxia on Pulmonary Circulation: Reflex Pulmonary Vasoconstriction, Am. J. Physiol. **189**: 253, 1957.
8. Lewis, B. M., and Gorlin, R.: Effects of Hypoxia on Pulmonary Circulation of the Dog, Am. J. Physiol. **170**: 574, 1952.
9. Stroud, R. C., and Rahn, H.: Effect of O_2 and CO_2 Tensions Upon the Resistance of Pulmonary Blood Vessels, Am. J. Physiol. **172**: 211, 1953.
10. Heemstra, H.: The Development of an Increased Pulmonary Vascular Resistance by Local Hypoxia, Quart. J. Exper. Physiol. **39**: 83, 1954.
11. Nahas, G. G., Visscher, M. B., Mather, G. W., Haddy, F. J., and Warner, H. R.: Influence of Hypoxia on the Pulmonary Circulation of Nonnarcotized Dogs, J. Appl. Physiol. **6**: 467, 1954.
12. Lanari-Zubiaur, F. J., and Hamilton, W. F.: Effect of Unilateral Anoxia on Pulmonary Circulation, Circulation Res. **6**: 289, 1958.
13. Rodbard, S., and Harasawa, M.: The Passivity of the Pulmonary Vasculature in Hypoxia, Am. Heart J. **57**: 232, 1959.
14. Smith, D. J., and Coxe, J. W.: Reactions of Isolated Pulmonary Blood Vessels to Anoxia, Epinephrine, Acetylcholine and Histamine, Am. J. Physiol. **167**: 732, 1951.
15. Ferguson, D. J., Berkas, E. M., and Varco, R. L.: Circulatory Factors Contributing to Alterations in Pulmonary Vascular Histology, S. Forum Am. College Surgeons **4**: 267, 1953.
16. Ferguson, D. J., Berkas, E. M., and Varco, R. L.: Experimental Methods for the Production of Pulmonary Hypertension *in* Pulmonary Circulation, edited by W. Adams, and I. Veith, New York, N. Y., 1959, Grune & Stratton, pp. 126-137.
17. Muller, W. H., Dammann, J. F., and Head, W. H.: Changes in the Pulmonary Vessels Produced by Experimental Pulmonary Hypertension, Surgery **34**: 363, 1953.
18. Lancaster, J. R., Harrison, R. W., Gold, R., and Moulder, P. V.: Effect of Systemic—Right Atrial Shunts on the Pulmonary Circulation. (To be published.)
19. Boake, W. C., Daley, R., and McMillan, I. K.: Observations on Hypoxic Pulmonary Hypertension, Brit. Heart J. **21**: 31, 1959.
20. Rattenborg, C.: Anestesiventil: Ventil til cirkelsystem og kunstig ventilation uden genånding, Ugesk. læger **118**: 1168, 1956.
21. Moulder, P. V., Eichelberger, L. E., and Roma, M.: Histochemical Characterization of Heart and Skeletal Muscle During Growth, A. M. A. Arch. Surg. **75**: 165, 1957.
22. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry (Methods), Baltimore, 1931, Williams & Wilkins Co., vol. 2.
23. Smith, F. J. C., Bennett, G. A., Heim, J. W., Thompson, R. M., and Drinker, C. K.: Morphological Changes in the Lungs of Rats Living Under Compressed Air Conditions, J. Exper. Med. **56**: 79, 1932.
24. Bennett, G. A., and Smith, F. J. C.: Pulmonary Hypertension in Rats Living Under Compressed Air Conditions, J. Exper. Med. **59**: 181, 1934.

(For Discussion, see page 631)

TREATMENT OF BANK BLOOD BY RESINS

Edward D. McLaughlin, M.D. (by invitation), Thomas F. Nealon, Jr., M.D., and John H. Gibbon, Jr., M.D., Philadelphia, Pa.

THE successful application of cation exchange resins to reduce abnormally high levels of ammonium and potassium in stored blood has been reported by Schechter, Nealon, and Gibbon.[6] Blood so treated still has an abnormally low pH, chiefly because of citrate preservative. By passing bank blood over an anion exchange resin, McLaughlin, Nealon, and Gibbon[4] were able to return pH and elevated anion concentrations to normal by substituting bicarbonate for the citrate and other increased anions.

The successful use of extracorporeal circulation in the treatment of various diseases in human beings has necessitated obtaining large quantities of compatible blood to prime the apparatus. Blood for this purpose is usually drawn within 24 hours of the scheduled time of operation. A number of previously typed and crossmatched donors therefore have to assemble at one time and place to donate this priming blood. If stored bank blood could be safely used, the collection of blood for open-heart operations would be greatly facilitated. The purpose of this study was to determine whether the electrolyte concentrations of canine bank blood could be restored to approximately normal values by passage over both an anion and a cation exchange resin. The safety of using this resin-treated blood was then tested by means of exchange transfusions in normal dogs.

METHODS AND MATERIALS

Donor blood was obtained by exsanguinating healthy adult mongrel dogs. ACD solution, Formula A, 75 ml. per 480 ml. of whole blood, was employed as the preservative. The 75 ml. of Formula A contains 1.64 Gm. trisodium citrate, 0.6 Gm. citric acid, and 1.84 Gm. dextrose (USP). Eight donor pools, each containing 3,000 ml. of whole blood, were stored in siliconized, sterile bottles at 5° C. Each pool was composed of blood from 3 or 4 different dogs. Four pools were stored for 14 days, and 4 for 21 days. A heparinized sample of blood from each dog was analyzed for blood pH, plasma hemoglobin, and

Department of Surgery, Jefferson Medical College, Philadelphia, Pa.
Supported in part by U. S. Public Health Service Grants, H3349 (C1) and H4486.
Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

electrolyte and metabolite concentrations. An aliquot of each pool was similarly analyzed before and after storage and after traversing a column of anion and cation exchange resins. Analytic methods have been given in detail in the previous papers.[4, 6]

Eighteen milligrams of heparin sodium was added to each 500 ml. of whole blood when it was removed from storage. The blood was then passed over the resin columns at a constant rate during a period of 30 to 35 minutes. Each 500 ml. of blood traversed 100 grams of the anion exchange resin, Dowex 1 × 8, converted from the chloride cycle to the bicarbonate-chloride cycle,[4] and 50 grams of the cation exchange resin, Dowex 50 × 8, converted from the sodium cycle to the sodium-calcium cycle. After passage over the resins the blood was then given as exchange transfusions to normal dogs.

The recipient animals weighed 7 to 9 kilograms. Blood for analysis was drawn from each dog in a heparinized syringe before starting the transfusion. The exchange transfusion was given during a 60-minute interval with the animals anesthesized with thiopental sodium. Fifty milliliters of blood was withdrawn from the dog's femoral artery by syringe and an equal amount of the pool blood was injected into the femoral vein. This process was repeated until the dog had received 200 per cent of its calculated blood volume, or 20 per cent of its body weight[1] in pool blood. The dog's blood was analyzed 2 hours and 24 hours after transfusion. The animals were observed for 2 weeks after the transfusion; they were then sacrificed and examined for gross evidence of hemorrhage, infection, emboli, and intravascular thrombosis.

RESULTS

The average analytic values obtained before storage, after storage, and after traversing the exchange resins on the four pools of 14-day blood are given in Table I. The same studies on 21-day blood are presented in Table II. The additional week of storage resulted in significant changes only in potassium and ammonium, both of which increased. The changes occurring in the substances analyzed are discussed below.

pH.—When the ACD solution was added to fresh blood, there was an immediate drop of pH to about 6.8. A further decline to less than 6.2 occurred during storage. After the blood traversed the resins, the pH approximated 7.10.

Sodium.—The addition of ACD solution to fresh blood elevated the concentration of sodium in the plasma to about 160 mEq./L. The value did not change during storage. Passage over the cation exchange resin restored this elevated value to the normal range.

Chloride.—The drop in chloride concentration on the addition of ACD solution can be accounted for by the dilution of the plasma. The further decline in concentration during storage may have been due to the passage of chloride into cells. No change in concentration occurred after passage over the anion resin.

TABLE I. CHANGES IN CANINE BLOOD STORED AT 5° C. FOR 14 DAYS
(AVERAGE OF 4 POOLS OF BLOOD OF 3 L. EACH)

	pH BLOOD	Na PLASMA (MEQ./L.)	Cl PLASMA (MEQ./L.)	K PLASMA (MEQ./L.)	Ca PLASMA (MG./100 ML.)	NH_4 BLOOD (MCG./100 ML.)	CITRATE PLASMA (MG./100 ML.)	PO_4 PLASMA (MG./100 ML.)	LACTATE BLOOD (MG./100 ML.)	PYRUVATE BLOOD (MG./100 ML.)	Hb PLASMA (MG./100 ML.)
Blood											
0 Day											
Heparin	7.37	146	117.9	4.1	9.6	136.6	1.7	3.76	26.6	1.66	9.06
After addition ACD solution	6.83	164	100.8	3.5	2.5	117.8	431.6	3.15	24.2	1.50	14.5
14 Day											
Storage at 5° C.	6.17	168	80.7	16.9	1.9	367.5	442.4	7.52	155.3	7.73	62.0
After passage over resins	7.07	136	82.9	0.3	4.5	116.5	3.5	2.52	58.6	0.8	70.1

TABLE II. CHANGES IN CANINE BLOOD STORED AT 5° C. FOR 21 DAYS
(AVERAGE OF 4 POOLS OF BLOOD OF 3 L. EACH)

	pH BLOOD	Na PLASMA (MEQ./L.)	Cl PLASMA (MEQ./L.)	K PLASMA (MEQ./L.)	Ca PLASMA (MG./100 ML.)	NH_4 BLOOD (MCG./100 ML.)	CITRATE PLASMA (MG./100 ML.)	PO_4 PLASMA (MG./100 ML.)	LACTATE BLOOD (MG./100 ML.)	PYRUVATE BLOOD (MG./100 ML.)	Hb PLASMA (MG./100 ML.)
Blood											
0 Day											
Heparin	7.33	136	117.2	4.1	9.2	133.8	1.3	3.64	10.9	1.08	10.6
After addition ACD solution	6.72	160	104.4	3.9	1.26	128.2	464.4	3.46	10.3	0.98	17.5
21 Day											
Storage at 5° C.	6.14	160	80.6	22.0	1.38	455.3	456.6	7.05	159.1	6.8	35.9
After passage over resins	7.14	137	84.9	0.9	4.7	136.3	1.6	3.07	68.8	1.73	52.6

Potassium.—Potassium concentration in plasma of bank blood increased progressively to an average of 17 mEq./L. after 14 days and 22 mEq./L. after 21 days of storage. After passage over the cation exchange resin the markedly elevated levels were reduced to less than 1.0 mEq./L.

Calcium.—The addition of ACD solution reduced the plasma calcium level, as measured by the technique employed, to approximately 25 per cent of normal. Since the calcium was not removed it must have been bound in unmeasurable form by the citrate. After the removal of the citrate by the anion exchange resin the calcium concentration was only 50 per cent of the original level. The present data do not account for the remainder of the calcium.

Ammonium.—Blood ammonium content increased progressively during storage so that after 3 weeks the concentration had approximately tripled. Reduction to the normal range occurred after passage over the cation exchange resin.

Citrate.—When ACD solution was added to fresh blood the plasma citrate concentration rose from an insignificant amount to approximately 450 mg. per 100 ml. The concentration did not change during storage. Passage of the blood over the anion exchange resin reduced the citrate content to normal.

Phosphate.—The phosphate concentration in the plasma after storage was about double that in fresh blood. After passage over the anion resin, the concentration returned to the normal range.

Lactate.—The lactate content of the blood rose markedly during storage. Passage over the anion resin removed the greater part of this increase, but did not reduce the concentration of lactate to the normal range.

Pyruvate.—After storage, the pyruvate content of blood was five or six times the pre-storage level. After traversing the anion resin, the pyruvate concentration returned to normal.

Plasma Hemoglobin.—During storage there was an increase in plasma hemoglobin. Passage of the blood over both resin columns resulted in slight increases in plasma hemoglobin.

To recapitulate, the addition of ACD solution to blood produced some increase in plasma sodium, a large increase in citrate with a concomitant decrease in ionizable plasma calcium and a marked drop in pH. With storage there was an increase in ammonium, potassium, phosphate, lactate, and pyruvate, and a decrease in plasma chloride with a further drop in pH. After passage over the resins the electrolyte concentrations returned to normal values except for potassium, calcium, and chloride (which were low), and lactate (which was high). There was a marked rise in pH due to the removal of the citrate and accumulated anions.

Eight dogs were given exchange transfusions of the resin-treated blood. None showed ill effects from the transfusion. When the animals were sacrificed 14 days after the exchange transfusion, postmortem examination showed no gross evidence of infection, hemorrhage, thrombosis, emboli, or infarction.

TABLE III. POOL "D" FOR EXCHANGE TRANSFUSION WITH DOG "D"

	pH BLOOD	Na PLASMA (MEQ./L.)	Cl PLASMA (MEQ./L.)	K PLASMA (MEQ./L.)	Ca PLASMA (MG./100 ML.)	NH_4 BLOOD (MCG./100 ML.)	CITRATE PLASMA (MG./100 ML.)	PO_4 PLASMA (MG./100 ML.)	LACTATE BLOOD (MG./100 ML.)	PYRUVATE BLOOD (MG./100 ML.)	Hb PLASMA (MG./100 ML.)
Blood											
0 Day											
Heparin	7.38	153	118.8	3.6	10.0	149.3	2.4	3.87	31.0	1.57	8.2
After addition ACD solution	6.81	163	108.6	3.6	2.2	141.2	430.3	3.21	28.8	1.52	19.7
14 Day											
Storage at 5° C.	6.16	175	88.6	16.8	2.1	371.5	435.8	8.23	186.7	4.25	52.5
After passage over resins	7.11	139	94.2	0.3	4.5	139.0	3.5	2.3	65.2	0.36	67.4
Dog D											
Before transfusion	7.30	167	122.9	4.2	9.1	137.7	1.8	3.63	23.0	1.06	19.9
2 hr. after transfusion	7.31	153	110.2	3.4	8.5	145.3	2.4	3.87	55.2	2.20	49.9
24 hr. after transfusion	7.34	147	118.8	3.6	10.0	149.8	3.5	3.52	31.0	0.57	8.21

Blood analyses in a typical experiment in which a dog was given an exchange transfusion of pooled blood stored for 2 weeks are shown in Table III. The same studies in another animal given exchange transfusion of blood stored for 3 weeks are shown in Table IV. Part of these changes are shown graphically in Fig. 1.

The changes in these individual pools of blood are similar to the average changes listed in Tables I and II. The recipient dog's blood prior to transfusion was similar to the freshly drawn heparinized pooled blood. The stored pooled ACD blood after it was treated by resins had a low potassium, calcium,

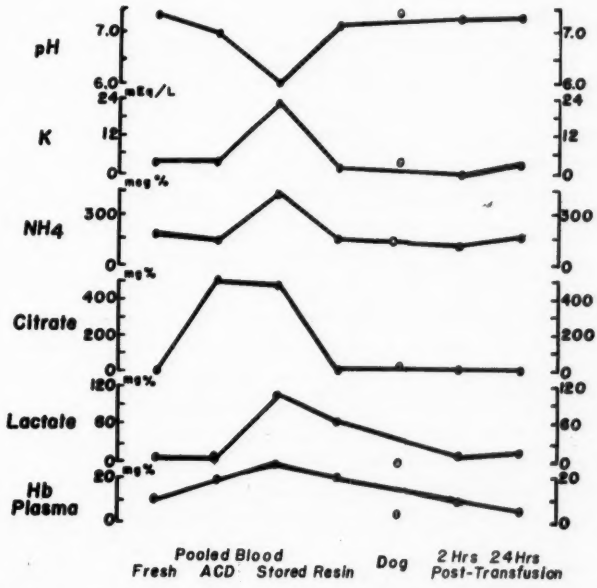


Fig. 1.—Changes which occurred in pH, potassium, ammonium, citrate, lactate, and plasma hemoglobin during storage for 21 days, passage over resins, and after exchange transfusion. The open circles are the levels in the blood of the recipient dog.

and chloride, and an elevated lactate with a resultant low pH. Two hours after transfusion all values were similar to those of the dog before transfusion except the lactate which was elevated but lower than in the pooled blood. Twenty-four hours after transfusion all values were normal.

DISCUSSION

ACD solution is used to preserve blood in almost all blood banks. The blood stored in ACD solution in glass bottles is generally discarded after 21 days' storage at 5° C. Less than 70 per cent of the cells retain full functional capacity after infusion if such blood is stored for longer periods. If ACD blood is stored in plastic containers, 70 to 80 per cent viability has been demonstrated after 25 days.[5]

TABLE IV. POOL "F" FOR EXCHANGE TRANSFUSION WITH DOG "F"

	pH BLOOD	Na PLASMA (MEQ./L.)	Cl PLASMA (MEQ./L.)	K PLASMA (MEQ./L.)	Ca PLASMA (MG./100 ML.)	NH_4 BLOOD (MCG./100 ML.)	CITRATE PLASMA (MG./100 ML.)	PO_4 PLASMA (MG./100 ML.)	LACTATE BLOOD (MG./100 ML.)	PYRUVATE BLOOD (MG./100 ML.)	Hb PLASMA (MG./100 ML.)
Blood											
0 Day											
Heparin	7.36	139	108.3	4.1	9.7	147.9	1.2	3.8	9.2	1.07	10.6
After addition ACD solution	6.91	158	90.3	4.0	0.9	143.9	453.0	3.6	9.0	1.01	19.1
21 Day											
Storage at 5° C.	6.01	161	75.1	22.1	1.1	437.6	447.9	6.5	109.2	6.5	24.2
After passage over resins	7.14	130	77.5	1.5	3.9	145.9	1.2	3.4	60.5	1.8	21.8
Dog F											
Before transfusion	7.38	144	116.6	3.9	8.9	143.3	2.4	4.27	5.0	1.22	4.7
2 hr. after transfusion	7.30	139	108.3	4.1	8.3	137.9	1.4	3.80	9.2	1.07	10.6
24 hr. after transfusion	7.36	147	123.0	3.4	9.3	148.8	1.3	4.14	22.2	1.22	5.9

Blood stored in citrate-phosphate-dextrose (CPD) solution was studied by Gibson, Rees, McManus and Scheitlin in 1957.[2] They reported more than 70 per cent viability of infused cells after 28 to 32 days' storage at 4° C. when the blood was contained in a plastic bag and infused in plastic recipient sets. A recent report indicates that multiple transfusions of such blood can be given to human beings without significantly elevating the plasma phosphate levels.[3] The CPD solution has the further advantage that it alters the pH less than does the ACD solution. It drops the pH to about 7.2 as compared to 6.8 to 7.0 with ACD solution. Previous work in this laboratory demonstrated that there is an even greater accumulation of potassium and ammonium when CPD solution is used. However, passage over a cation exchange resin will remove the excess potassium and ammonium.

Heparinized blood used to prime heart-lung machines is collected within 24 hours of operation. After 48 hours, heparinized blood tends to develop tiny clots. ACD-preserved blood with its high concentrations of potassium, ammonium, and citrate cannot be used. Smith, Brown, Young, and Sealy[7] have successfully primed a heart-lung machine with blood stored up to 5 days using Edglugate Mg as a preservative. Beyond that time, increased potassium and depression of antihemophilic globulin and factor V levels contraindicate its use.

Since our animals tolerated exchange transfusions of ACD blood treated by ion exchange resins with no ill effects, it is possible that such blood might prove satisfactory for priming heart-lung machines. Stored blood so treated must be tested in the extracorporeal apparatus to determine the period of storage permissible for animals and for man. If it proves safe to use ordinary bank blood for this purpose, collection of blood for open cardiac operations would be greatly simplified. Furthermore, bank blood would be available for emergency use of the heart-lung machine.

SUMMARY

1. Altered levels of blood pH and metabolites and of plasma electrolytes which are present in stored ACD blood may be restored to approximately normal values after passage over anion-cation exchange resins.

2. Canine ACD blood stored 2 to 3 weeks and then treated by anion-cation exchange resins and used for exchange transfusions in dogs produced no detectable harmful effects to the recipients.

REFERENCES

1. Challet, H. A., Clauss, R. H., Giannini, S. G., and MacFee, W. F.: A Safe and Simple Method of Blood Collection for Transfusion Utilizing a Cation Exchange Resin, Ann. Surg. **147**: 69-74, 1958.
2. Gibson, J. G., II, Rees, S. B., McManus, T. J., and Scheitlin, W. A.: Citrate-Phosphate-Dextrose Solution for Preservation of Human Blood, Am. J. Clin. Path. **28**: 569-578, 1957.
3. Gibson, J. G., II, Rees, S. B., and McManus, T. J.: Replacement of Blood Loss During Surgical Procedures With Blood Collected in Citrate Phosphate Dextrose Solution, New England J. Med. **262**: 595-597, 1960.

4. McLaughlin, E. D., Nealon, T. F., Jr., and Gibbon, J. H., Jr.: The Removal of the Citrate Additive and Accumulated Anions From Bank Blood. (To be published.)
5. Sack, T., Gibson, J. G., and Buckley, E. S., Jr.: The Preservation of Whole ACD Blood Collected, Stored, and Transfused in Plastic Equipment, Surg. Gynec. & Obst. **95**: 113-119, 1952.
6. Schechter, D. C., Nealon, T. F., Jr., and Gibbon, J. H., Jr.: The Removal of Excessive Potassium and Ammonium From Bank Blood Prior to Transfusion, Surg. Gynec. & Obst. **108**: 1-6, 1959.
7. Smith, W. W., Brown, I. W., Jr., Young, W. G., Jr., and Sealy, W. C.: Studies of Edglugate-Mg: A New Donor Blood Anticoagulant-Preservative Mixture for Extracorporeal Circulation, J. THORACIC SURG. **38**: 573-585, 1959.

(For Discussion, see page 631)

GAS-EXCHANGE DYNAMICS OF GLYCEROLIZED FROZEN BLOOD

Thomas G. O'Brien, Lieutenant (MC) USNR (by invitation), Chelsea, Mass., and Elton Watkins, Jr., M.D., Boston, Mass.

CLINICAL experience has demonstrated to us that blood treated with glycerol and stored for prolonged periods of time in the frozen solid state may be used, on thawing, as a satisfactory total blood volume replacement during cardiovascular surgery and extracorporeal circulation.[18] This finding has resulted in a considerable improvement in the logistics of blood procurement and increased flexibility in tailoring blood transfusion requirements to the needs of the individual patient.

Basic studies of the glycerolization technique by Tullis and associates of the Protein Foundation, Inc., demonstrated that human red blood cells could be stored at −80 or −120° C. for periods up to 44 months.[24, 25] When such cells were thawed and transfused, their survival in the circulation was comparable to that of freshly drawn cells treated by conventional blood bank techniques. Prior to the freezing process, it was necessary to separate cells from plasma and to treat the cellular elements of the blood with glycerol to minimize the formation of large ice crystals (which might disrupt the cell membrane) to prevent increased ionic concentrations within the cell as water was transformed to ice, and to minimize extracellular diffusion of important cell constituents.[1, 14, 21] After thawing, the glycerol was removed from the cells by washing and the cells were reconstituted in their own plasma or a suitable suspension medium containing purified albumin solution. The various manipulations of cell separation and introduction and removal of glycerol from the cells require a modification of the fractionating centrifuge originally devised by Cohn for separation of cellular and protein fractions of fresh blood (Fig. 1).[2, 24, 25] Since glycerol, the cellular elements of the blood, and the various plasma components have varying specific gravities, centrifugation affords a method for separating blood elements or for the introduction or removal of glycerol from the separated cellular elements. Cell destruction is minimal during processing and the closed system ensures complete asepsis.

From the Blood Preservation and Surgical Research Laboratories of the U. S. Naval Hospital, Chelsea, Mass., the Department of Surgery, Lahey Clinic, and the Surgical Service, New England Deaconess Hospital, Boston, Mass.

Aided by grants from the Office of Naval Research, the Lahey Foundation, the National Heart Institute, U. S. Public Health Service (H-3803), and the American Proctologic Research Foundation.

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

In 1956, The Blood Preservation Laboratory was established at the U. S. Naval Hospital, Chelsea, Massachusetts, to evaluate the merits of the glycerolization-freezing method at a clinical level.[22] Haynes and associates[7] soon established the value of the method in over 1,000 transfusions. In addition to the obvious advantages of blood stock-piling without the necessity for discarding outdated blood, the over-all reaction rate was found to be only one tenth of that seen following conventional transfusion of blood preserved at 4° C. in acid-

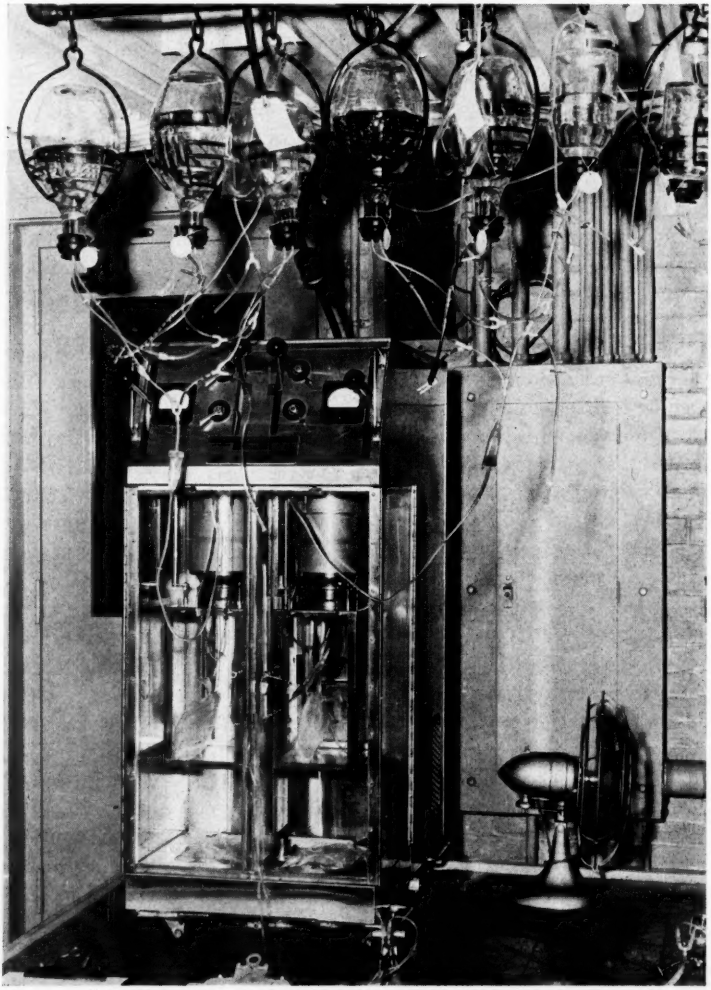


Fig. 1.—View of the Cohn A. D. L. blood fractionator during glycerolization of blood. Wash solutions are suspended above the unit. The interchangeable sterile cartridge unit bearing the rotating centrifuge bowl and collection bags is located within a refrigerated compartment. The centrifuge bowl connects to the drive mechanism by a chuck attachment. Drive controls are located above the spinning bowl.

citrate-dextrose solution. In view of this experience it seemed advisable to test blood passed through the glycerolization process as replacement in the massive amounts required so frequently for cardiac and thoracic surgery. Such a study was given added impetus by the finding in our laboratory that heparinized blood treated by the glycerolization process maintains full heparin activity up to 7 months' storage at –80° C. (the longest period studied).[19] It seemed possible that heparinized blood could be stock-piled without time limitation and then used as a medium for pump-oxygenator priming and transfusion during open-heart or vascular surgery.

Basic to such an application was the need for confirmation of the clinical observation that glycerolization did not alter hemoglobin structure or intracellular environment so that the ability of the red blood cell to transport gases was seriously impaired. Certain properties of oxygen transport by the red blood cell are expressed by the oxyhemoglobin dissociation curve, which equates values of oxyhemoglobin saturation (the percentage of total hemoglobin in

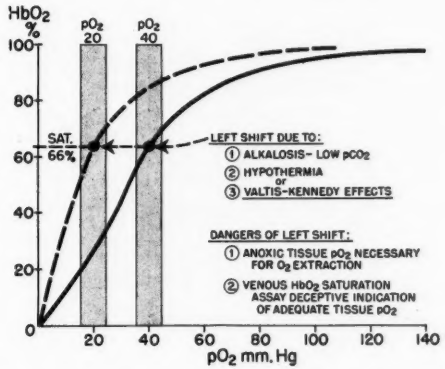


Fig. 2.—Diagrammatic representation of consequences of a shift to the left of the normal oxyhemoglobin dissociation curve *(solid line)*.

the oxygenated state) to the partial pressure of oxygen in a gas mixture in equilibrium with the blood. Construction of an oxyhemoglobin dissociation curve for a given blood specimen enables one to draw conclusions as to the manner in which that blood will give up oxygen within the tissues to provide an oxygen pressure within the tissues for adequate aerobic cellular metabolism.

Of particular significance in the present study was the finding by Valtis and Kennedy[27] that blood collected in the usual acid-citrate-dextrose solution demonstrated an impairment of oxyhemoglobin dissociation after a number of days' storage at 4° C. The character of the changes demonstrated by them are illustrated diagrammatically in Fig. 2. As a result of a shift to the left of the oxyhemoglobin dissociation curve, the tissue oxygen concentration or partial pressure (pO_2) is in danger of falling to anoxic levels before an adequate dissociation of oxygen from hemoglobin may occur.

The curve shift gradually reversed once the transfused blood was within the body, but large volume blood replacements resulted in serious impairment

of tissue oxygen tension persisting for several days. At certain times, the tissue oxygen tension could be actually worsened by transfusion despite apparent correction of an anemic state. The implications of such a situation are obvious in thoracic surgery, where the derangement may be compounded by diminished cardiac output and ventilatory reserve.

The Valtis-Kennedy effect is related to variations of blood electrolyte concentrations and may be eliminated by addition of sodium chloride to plasma.[26] Since the glycerolization process (unless carefully controlled) might be expected to derange just these electrolyte concentrations, a determination of oxyhemoglobin dissociation properties was necessary to confirm our impression that a serious alteration of hemoglobin chemistry was not induced by the glycerolization process.

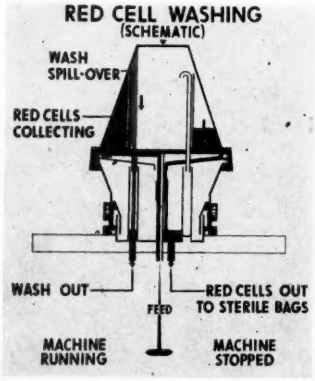


Fig. 3.—Diagrammatic representation of the centrifuge bowl used for cell separation and washing.

METHODS

Glycerolization and Freezing of Blood.—

Blood from a healthy young donor is collected by phlebotomy, drawing 475 ml. into a plastic bag containing either acid-citrate-dextrose solution or heparin-saline solution. The citrate bag contains 75 ml. of acid-citrate-dextrose solution, National Institutes of Health Formula A. The heparin bag contains 2,000 USP units of heparin sodium in 30 ml. of 0.89 per cent sodium chloride solution buffered to pH 7.4. Between collection and glycerol processing, the bags are stored at 4° C. for periods up to 5 hours.

Blood is glycerolized and deglycerolized by passage through a centrifuge of the Cohn type (Cohn A.D.L. fractionator*) (see Fig. 1). Interchangeable cartridge units are placed within a refrigerated compartment of the fractionator and the centrifuge portion of the cartridge assembly rotated at a revolution rate which may be varied depending upon the type of separation desired. A diagram of the Type II centrifuge bowl (Fig. 3) used for glycerolization and deglycerolization illustrates the manner of introduction of blood and withdrawal of blood fractions. Blood or washing solutions are admitted by pressure flow through a plastic tube manifold. After passage through the centrifuge bowl, the separated elements are collected in plastic bags connected to outlet ports in the base of the bowl. Since the collecting bags and centrifuge bowl are assembled and sterilized as an

*Arthur D. Little Co., Cambridge, Mass.

interchangeable cartridge unit, the dangers of contamination during attachment of tubing connections are eliminated.

In the glycerolization phase, blood is forced onto the rotating bowl at a flow rate of approximately 50 ml. per minute. The bowl is rotated to give a gravitational force of 425 g. The blood passes to the periphery of the bowl by centrifugal force. Separation of the blood into a heavier red cell layer at the bowl periphery and an inner lighter plasma layer occurs as the bowl fills to its capacity of 350 ml. The separated plasma accumulates in the inner area of the bowl and overflows a central weir into a plastic bag. After the entire unit of blood has been infused and the maximal amount of plasma collected, the plasma bag is sealed and detached from the cartridge unit for freezing. Without interruption, a large volume of 20 per cent solution of glycerol is introduced into the bowl followed by a large volume of 50 per cent glycerol. As each successive glycerol solution increases in volume within the centrifuge, it traverses the packed cell residue contained within the bowl from the periphery to the central weir of the bowl and thence spills over into a discard outlet. The plasma remaining in the bowl at the end of the cell separation cycle dilutes the initial portions of 20 per cent glycerol entering the bowl and a similar dilution occurs as the glycerol solution concentration is increased. Glycerol equilibration between the cells and wash solution thereby progresses gradually by means of counter-current dialysis. Following addition of the 50 per cent glycerol solution, the centrifuge is stopped, permitting the glycerolized cells to flow into a separate plastic bag which is sealed and detached.

The plasma and red cell bags are marked and placed in a cardboard container for storage in a −80° or −120° C. freezing unit. Temperatures prevailing within the freezers are continuously recorded. An automatic alarm system signals any possible failure of the freezer mechanism. Temperature equilibration is obtained within 6 to 8 hours after the bags are placed within the freezer.

To reconstitute the blood, the frozen units are thawed for 10 minutes in a rotating water bath at 37° C. Glycerol is removed from the cell mass by exposure within the centrifuge to solutions containing decreasing concentrations of glycerol. This dialysis must progress gradually since water will enter the cell four times faster than the glycerol will leave and a slow step-wise progression must be used to prevent hemolysis. A small amount of plasma is washed through to displace the last wash solution. The cell mass is then removed into a plastic administration bag where it can be reconstituted with its own plasma or other desired suspension medium. The reconstituted blood is stored at 4° C. until ready for use.

It now takes us 35 minutes to glycerolize a unit of blood and 45 minutes to reconstitute it. With the present equipment one technician can process four units simultaneously. Glycerol concentration is 40 per cent within the frozen cell and less than 1 per cent following reconstitution. To meet special clinical requirements, the electrolyte concentration and pH of the reconstituted blood may be varied at will by altering the composition of the final washing solutions.

Blood and Gas Analysis Methods.—

Blood samples were withdrawn from patients by arterial or venous puncture. During in vitro studies, plastic blood bags were inverted 25 times to assure adequate mixing. Blood was withdrawn for sampling or stored after equilibration in Luer-Lok syringes lightly lubricated with mineral oil. Syringe dead space was filled with 1 per cent heparin solution in saline. Following collection of a sample, the syringe was sealed with mercury, capped with a locking hub, and immediately immersed in an ice bath.

Samples of blood 6.0 ml. in volume were equilibrated in Barcroft tonometers of approximately 325 ml. capacity by rotation for 20 minutes in a 37° C. water bath, following gassing of the tonometer for 30 seconds at 3 L. per minute from cylinders containing varying proportions of nitrogen, oxygen, and carbon dioxide. The carbon dioxide tension was approximately 40 mm. Hg in each cylinder and the ratio of nitrogen to oxygen varied to give approximate oxygen tensions of 15, 25, 30, 60, 90, or 120 mm. Hg.

Blood oxygen capacity as determined by the method of Van Slyke and Neill[17] was compared with the spectrophotometric method of Hickam and Frayser,[8] using a Beckman spectrophotometer Model DU. Published density coefficients were confirmed by the standardization in a large number of normal bloods against the manometric capacity. Using our constants, 11 consecutive fresh blood samples showed a mean deviation between manometric and spectrophotometric values of −0.07 vol. per cent with a standard deviation of 0.49 vol. per cent. For 16 consecutive comparisons on samples of thawed deglycerolized ACD and heparinized blood, the mean deviation was −0.15 vol. per cent with a standard deviation of 0.40 vol. per cent. The lack of significant statistical variation between such comparisons of fresh and frozen blood oxygen capacities indicates the validity of our spectrophotometric standards and excludes the possibility of significant methemoglobin formation in the stored blood. There was no increased disparity between manometric and spectrophotometric capacity values in the older stored bloods, which ranged from 3 to 31 months' storage time.

Blood oxygen and carbon dioxide contents were determined initially by the combined manometric method of Van Slyke and Neill[17] using 1.0 ml. blood samples. Since the resulting oxyhemoglobin saturation values showed insignificant deviation from simultaneous oxyhemoglobin determinations by a spectrophotometric method, we abandoned the combined blood manometric analysis and relied in this study upon the quicker spectrophotometric method. During this later period, plasma carbon dioxide content was determined by the manometric method of Van Slyke and Neill[17] on 1.0 ml. plasma samples obtained after centrifugation under oil.

Blood hydrogen ion concentration was measured with the Beckman Model Gs pH meter with an external anaerobic electrode immersed in a 37° C. water bath. The electrode was standardized with pH 7.00 buffer solution. Microhematocrit determinations were made.[9]

Following tonometric equilibration, the gas phase within the chamber was analyzed for carbon dioxide and oxygen by duplicate determinations with the Scholander apparatus.[20] Gas tensions were computed from this analysis and the ambient barometric pressure corrected for water vapor tension.

Computation of Oxyhemoglobin Dissociation Curves.—

Oxyhemoglobin dissociation curves were constructed using the general principles of the Harvard Fatigue Laboratory as outlined by Dill in the report of Keys, Hall, and Barron.[11] The technique of computation is shown in Fig. 4. Generally between three and five observations with oxyhemoglobin saturations between 30 and 80 per cent were available to give a linear relationship between HbO_2/Hb (oxyhemoglobin/reduced hemoglobin) and corrected pO_2 when plotted on 2 by 3 cycle log-log paper. The resulting best-fit line was used to construct the conventional sigmoid dissociation curve comparing oxyhemoglobin saturation to corrected pO_2. In addition, two values were determined for each linear log-log plot to express the contour and position of the constructed linear sigmoid curve: (a) the pO_2 value where $HbO_2 = Hb$ or 50 per cent oxyhemoglobin saturation; and (b) the slope of the log-log line which represents the value "n" in the Hill-Barcroft equation expressing the general characteristics of oxyhemoglobin dissociation:

$$\frac{HbO_2}{Hb} = K\ (pO_2)^n$$

$$\text{or } \log HbO_2/Hb = \log K + n \log (pO_2)$$

The pO_2 50 per cent value represents the degree of shift of the dissociation curve to right or left of the standard position while the value "n" determines the degree of sigmoid inflection of the curve.

All pO_2 values were corrected to a standard pH_s of 7.40 by the equation:

$$\frac{\Delta \log pO_2}{\Delta pH} = K$$

Since there was no reason to suppose that the value of the constant K in this equation would be the same for degycerolized blood as for fresh blood, determinations of the constant

were carried out by construction of multiple dissociation curves on aliquots of deglycerolized acid-citrate-dextrose or deglycerolized heparinized blood in which the pH was altered widely by alterations of pCO_2, or addition of lactate or sodium bicarbonate. The value of K for deglycerolized heparinized blood was found to be −0.475, standard deviation 0.060 (eight determinations). The value of K for deglycerolized acid-citrate-dextrose blood was found to be −0.2945, standard deviation 0.0117 (seven determinations; pH 6.55 → 7.44). The standard value for K of −0.480 above 7.00 pH and −0.460 below 7.00 pH was used for fresh heparinized and ACD blood corrections.[27]

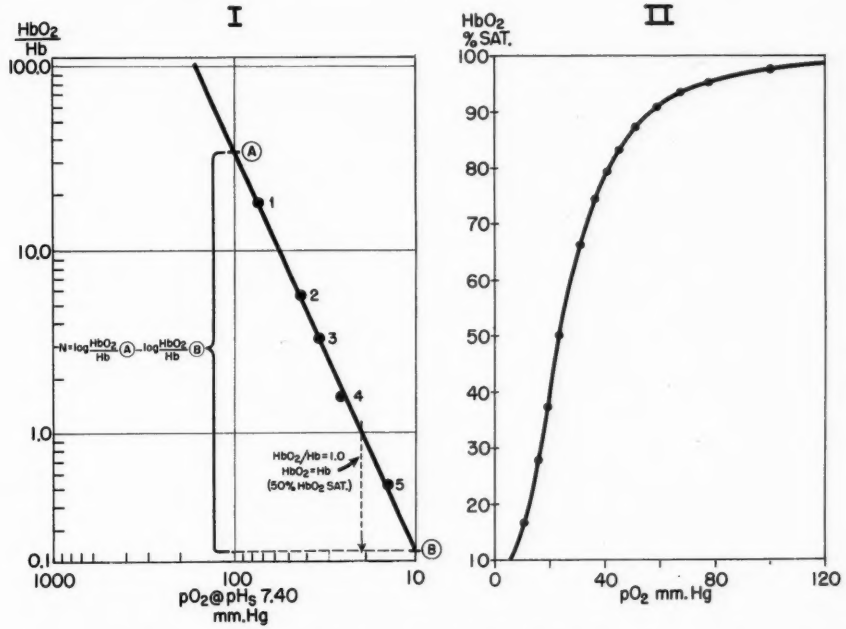


Fig. 4.—Method of construction of oxyhemoglobin dissociation curve. Between three and five observations are made equating oxyhemoglobin saturation to pO_2 at a given pH. The saturation value is converted to the ratio: HbO_2/Hb (oxyhemoglobin per cent/reduced hemoglobin per cent) and the pO_2 value corrected to a standard pHs 7.40 equivalent. These points are then plotted on log-log paper (I) to give a straight line. Values are computed for pO_2 at which oxyhemoglobin saturation is 50 per cent and "*n*" representing the slope of the line. Conventional dissociation curves are constructed (II) by reading off points upon the best-fit log-log line.

RESULTS

Standard Curves for Fresh Heparinized Blood From Healthy Donors and Preoperative Patients.—

To confirm the validity of our methods, six curves were constructed using fresh heparinized blood of healthy donors and patients preoperative for thoracic operation, without evidences of anemia, polycythemia, or arterial oxyhemoglobin desaturation. The conformity of the standard curve resulting from averaging these values is shown in Fig. 5. The pO_2 value for 50 per cent oxyhemoglobin saturation was 23.81 mm. Hg with a standard deviation of

3.15 mm. Hg. The value of "n" was 2.540 ± 0.13. The standard curve conforms closely to the curve of Morse[15] for adults. It is likely that the larger standard deviation for our pO_2 value results from the use of blood from an adolescent and two children in our standard curve computations. Since most of the blood donors in this series were healthy sailors in late adolescence, alterations of contour of the individual blood specimens from published adult standard curves might be expected.[3, 4, 15] Conclusions are based largely on the comparison of curves with control curves on that blood or changes in contour of the individual curves with the passage of time.

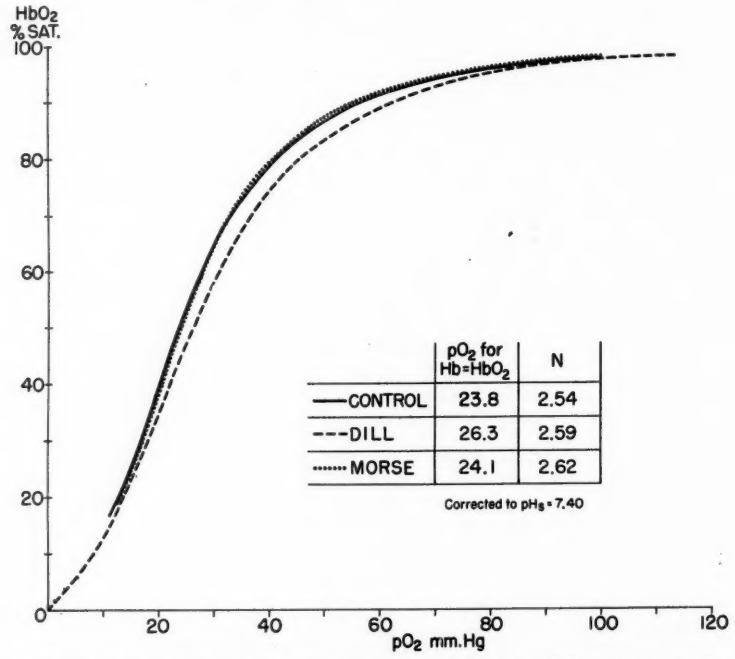


Fig. 5.—Comparison of our normal control oxyhemoglobin dissociation curve to the curves reported by Dill[3] and Morse.[15]

Fresh Acid-Citrate-Dextrose Blood Preserved at 4° C.—

Contrary to the findings of Valtis and Kennedy, we found a significant shift of oxyhemoglobin dissociation to the left on the first day of storage at 4° C. In two such units, pO_2-50 per cent values were 11.0 and 17.5 mm. Hg. Valtis and Kennedy could demonstrate significant shifts only after storage of such blood at 4° C. for periods of more than 7 days.[27] We demonstrated that the shift showed progression with prolonged storage at 4° C.—a finding consistent with the observations of Valtis and Kennedy.[27]

Blood Collected in Acid-Citrate-Dextrose Solution, Glycerolized, and Frozen at –80° C. for Periods up to 31 Months.—

It was impossible to detect a significant shift of the oxyhemoglobin dissociation curve in blood immediately upon reconstitution after –80° C. storage for

up to 31 months (Fig. 6). Although a slight deviation to the left from the normal curve was observed in four of five units studied, the pO_2-50 per cent value was altered less than 3.6 mm. Hg in these units, an insignificant change. Moreover, there appeared to be no consistent progression of the slight variation during successive months of –80° C. storage. It was not possible to compare dissociation curves observed before freezing with curves obtained on the same blood after storage since all units were glycerolized and frozen before the inception of this study.

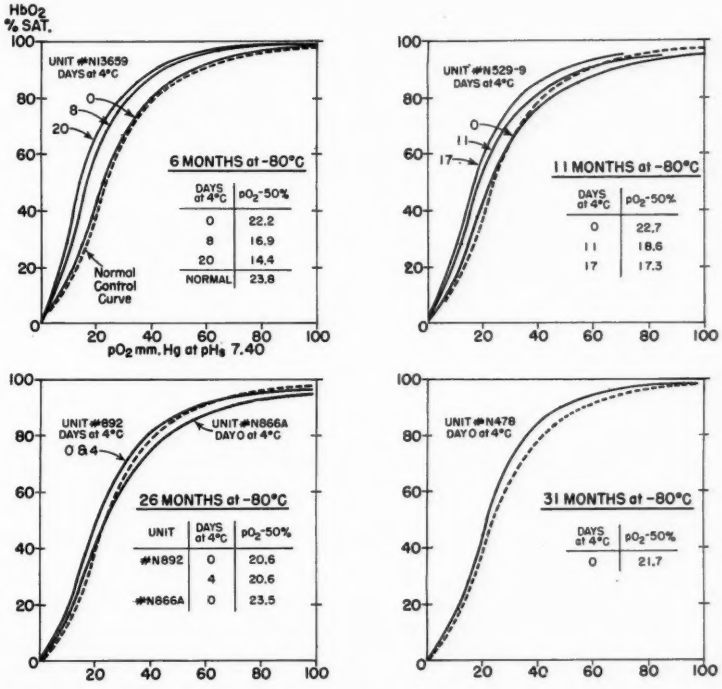


Fig. 6.—Oxyhemoglobin dissociation curves for blood collected in acid-citrate-dextrose solution and preserved for varying lengths of time at –80° C. After thawing and deglycerolization, curves were determined immediately and after several days' additional storage of the now fluid blood at 4° C. Although insignificant shifts are observed immediately upon thawing, the characteristic Valtis-Kennedy shift is observed as the blood is stored at 4° C.

The characteristic significant shift of dissociation to the left was observed, however, when these units were stored at 4° C. for periods of 8 days or over after deglycerolization and reconstitution. The magnitude of the shift at 4° C. did not appear to be greater than that observed by Valtis and Kennedy for fresh acid-citrate-dextrose blood stored at 4° C. for comparable lengths of time.[27]

Blood Collected in Heparin-Saline Solution, Glycerolized, and Frozen at –80° C. for Periods up to 7 Months.—

Table I indicates a significant shift of oxyhemoglobin dissociation to the left in curves determined immediately after reconstitution following collection

in heparin, glycerolization, and freezing. Since heparinized blood is not stored at 4° C. after reconstitution but is used immediately for pump priming and transfusion, studies were not conducted to indicate progression of the shift on prolonged storage. Attention is directed to the in vivo observation that the shift was corrected in a much more rapid manner following total blood volume replacement with heparinized deglycerolized blood than with fresh acid-citrate-dextrose blood volume replacement (Fig. 7). Despite the magnitude of the early shift observed in vitro, the clinical study carried out and demonstrated in Fig. 7 suggests that correction may be so prompt that shifts comparable to those demonstrated in vitro could not be shown.

Dynamics of Oxygen and Carbon Dioxide Exchange.—

The oxyhemoglobin dissociation curve expresses the static equilibrium conditions of oxygen transport and gives no indication as to the rate at which oxygen will combine with hemoglobin in the red cell. We have used a pump-oxygenator circuit to study these dynamic changes. In one experiment, our

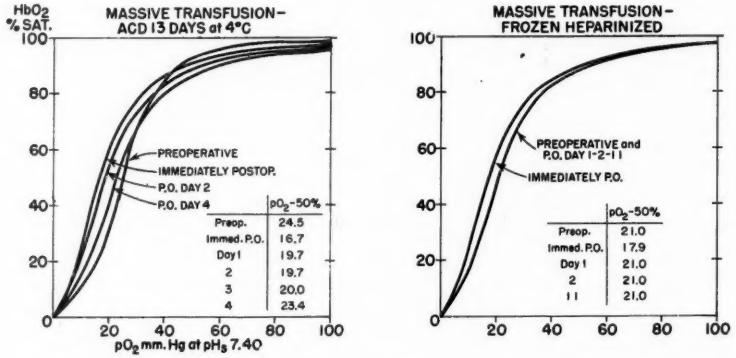


Fig. 7.—Studies in patients after total blood volume replacement with bank ACD blood stored by the conventional techniques at 4° C. for 13 days, as compared to similar replacement with blood which had been preserved in heparin and frozen at −80° C.

ACD 13 Days: A 47-year-old crane operator undergoing total cardiac bypass for operative treatment of aortic stenosis received 4,500 ml. of bank ACD blood late during the procedure. A pronounced left-shift has not completely corrected by the fourth day of his uncomplicated convalescence. (An 11-day curve was not suitable for computation because of gas analysis errors.)

Frozen Heparinized: A 13-year-old girl received 4,800 ml. of frozen deglycerolized heparin blood stored 5 to 12 days at −80° C. during total cardiac bypass for correction of pulmonic stenosis. Shift was observed immediately after operation, but curves drawn on postoperative days 1, 2, and 11 showed no significant deviation from the control curve. Neither patient showed bleeding tendencies nor late difficulties.

TABLE I

DAYS AT −80° C.	pO_2 FOR 50% HbO_2
2	17.2 mm. Hg
5-12	17.9
4	15.5
207	14.0
210	18.8
Mean and S.D.	16.3 ± 1.98
Normal Control Curves Mean and S.D.	23.8 ± 3.15

pump-oxygenator[28] was primed with fresh heparinized venous blood and the rate of oxygenation under standard conditions observed. In another experiment, deglycerolized heparinized blood was used in a similar manner. Since the oxyhemoglobin saturation changes were not comparable in the two experiments, direct comparisons are not possible but equation to published figures for bypasses indicates that no gross impairment of oxygenation rate is induced by the glycerolization process. In the recirculation experiment with fresh heparinized blood, we found that our disc oxygenator introduced into venous blood 2.88 ml. of oxygen per disc per minute in raising oxyhemoglobin saturation from 42 to 68 per cent. Gross and associates[6] found that their oxygenator introduced 3.0 ml. of oxygen per disc per minute at comparable saturation levels. Using deglycerolized heparinized blood (which had been stored 6 months at –80° C.) as a prime in our oxygenator, we found 2.22 ml. of oxygen per disc per minute was introduced to raise oxyhemoglobin saturation from 63 to 88 per cent. Gross and associates[6] reported an exchange of 2.0 ml. per disc per minute. Thus we conclude that no gross deficiencies in oxygen uptake dynamics exist following glycerolization of blood. Further studies are in progress to document precisely these dynamic changes as well as the conditions of carbon dioxide transport in the above experiment. The rate of change of blood pCO_2 did not significantly vary between the two types of blood.

DISCUSSION

We have confirmed the finding of Valtis and Kennedy[27] that the oxyhemoglobin dissociation curve shows a shift to the left as fresh acid-citrate-dextrose blood is preserved at 4° C. beyond 8 days. They concluded that the shift was dependent upon changes in intracellular electrolyte concentration and pH, and could be prevented by the addition of sodium chloride solution to the preserved blood to bring the final concentration of sodium chloride to 0.45 per cent.[26] The present deglycerolization technique does not result in significant shifts of the oxyhemoglobin dissociation curve during periods of storage of the citrated blood cell at –80° C. up to 31 months, despite the considerable alterations of electrolyte concentration which must occur during glycerolization and deglycerolization. However, we have demonstrated that shifts do occur early following reconstitution of heparinized deglycerolized blood. This aspect of the problem is under investigation since it is just this blood which should possess a normal oxyhemoglobin dissociation pattern at all times in view of its use in pump-oxygenator perfusions. Although the shift appears to be rapidly correctable once the blood is infused in the body, elimination of the effect would be desirable. At the present time, our washing solutions in the deglycerolization phase are designed to bring heparinized blood to a pH of 7.10, comparable to that of acid-citrate-dextrose blood. This pH has been shown to give maximal survival of red cells when blood is stored at 4° C. The use of large volumes of heparinized deglycerolized blood at this pH in

extracorporeal perfusions has not resulted in serious body acid-base disturbances but we would prefer to have our wash solutions adjusted in the future to allow for a more physiologic pH early during a perfusion. There is no necessity for keeping heparinized deglycerolized blood at a pH of 7.1 since it is not stored for long periods of time after reconstitution and hence should not necessarily meet the requirement of acidity indicated for reconstituted acid-citrate-dextrose blood. This latter blood after reconstitution is kept for days at 4° before conventional transfusion therapy while reconstituted heparinized blood is withdrawn from the freezer and used promptly on reconstitution. As a consequence, our present investigations are aimed at (a) adjusting final washing solutions in the deglycerolization phase to bring the thawed deglycerolized blood to a pH of 7.4, and (b) to increase the sodium chloride content of the washing solutions just sufficiently to eliminate the Valtis-Kennedy shift observed for frozen heparinized blood. It is hoped that the latter maneuver will eliminate the shift without producing serious derangements of plasma electrolyte concentrations in the final reconstituted blood package. The ability to perform these various adjustments easily and without loss of blood sterility is a great advantage of the glycerolization freezing process.

SUMMARY

1. Blood treated with glycerol and stored for prolonged periods of time at –80° C. may be used as a suitable medium for pump priming and transfusion in cardiovascular surgery. The glycerolization process requires the separation of cells from plasma and addition of glycerol to the cell fraction in a modified Cohn fractionator.

2. Blood collected in acid-citrate-dextrose solution and preserved at –80° C. for up to 31 months demonstrates no deviation of oxyhemoglobin saturation curve comparable to that which has been demonstrated for fresh acid-citrate-dextrose blood stored at 4° C. beyond 7 days by conventional blood banking techniques.

3. Blood collected in heparin-saline solution, glycerolized, and frozen shows a significant shift of the oxyhemoglobin dissociation curve to the left when reconstituted after 4 to 210 days' storage. On infusion of such blood into a patient as a total blood volume replacement, the shift is corrected in less than 1 day. Similar shifts induced by total blood volume replacement with bank ACD blood stored over 13 days at 4° were not corrected completely at the end of 4 days.

4. The rate of exchange of oxygen and carbon dioxide in a pump-oxygenator is the same for deglycerolized heparinized blood as for fresh heparinized blood.

5. Studies are in progress to adjust the technique of cell washing so that the addition of sodium chloride to the reconstituted heparinized blood will eliminate the oxyhemoglobin dissociation shift.

Lt. (jg) John W. Zemp collaborated with us and performed many of the analyses reported. We are also indebted to Capt. Lewis L. Haynes, Chief of Surgery, U. S. Naval

Hospital, Commander Mary V. Sproul, and Dr. James L. Tullis and associates (of the Protein Foundation, Inc.) for encouragement and advice. They are not responsible for any errors noted by the reader. This work would not have been possible without the assistance of J. A. Cavins, Lt. (MC) USNR, and the prolonged labor by our technicians in the Preservation Laboratory, B. C. Sutherland HM-2, USN, M. Bethea, HMC USN, and our pump technicians, Mr. Ralph Sommers and Mr. Joel Johns.

REFERENCES

1. Brown, I. W., Jr., and Hardin, H. F.: Recovery and In Vivo Survival of Human Red Cells; Studies of Red Cells After Storage up to 6¼ Months at Subzero Temperatures, A. M. A. Arch. Surg. **66**: 267-282, 1953.
2. Cohn, E. J.: Blood Collection and Preservation in American Association of Blood Banks. Proceedings, Fourth Annual Meeting, Minneapolis, Minn., Oct. 22-24, 1951.
3. Dill, D. B.: Handbook of Respiratory Data in Aviation, Washington, D. C., 1944, National Research Council.
4. Drabkin, D. L.: Aspects of the Oxygenation and Oxidative Function, *in* Haemoglobin: A Symposium Based on a Conference Held at Cambridge in June, 1948, in Memory of Sir Joseph Barcroft, edited by F. J. W. Roughton and J. C. Kendrew, New York, 1949, Interscience Publishers, Inc., pp. 36-45.
5. Gordy, E., and Drabkin, D. L.: A Simplified Spectrophotometric Technique Applicable to Standard Equipment for Determinations of O_2 Saturation of Blood, Proc. Soc. Am. J. Med. Sc. **221**: 231-232, 1951.
6. Gross, R. E., Sauvage, L. R., Pontius, R. G., and Watkins, E., Jr.: Experimental and Clinical Studies of a Siphon-Filling Disc Oxygenator System for Complete Cardiopulmonary Bypass, Ann. Surg. **151**: 285-302, 1960.
7. Haynes, L. L., Tullis, J. L., Pyle, H. M., Sproul, M. T., Wallach, S., and Turville, W. C.: Clinical Experiences With the Use of Glycerolized Frozen Blood, J. A. M. A. **173**: 1657-1663, 1960.
8. Hickam, J. B., and Frayser, R.: Spectrophotometric Determination of Blood Oxygen, J. Biol. Chem. **180**: 457-465, 1949.
9. Jones, A. R.: Device for Rapidly Deriving Hematocrit of Blood Centrifuged in Ungraduated Tubes, New England J. Med. **254**: 172-174, 1956.
10. Ketchel, M. M., Tullis, J. L., Tinch, R. J., Driscoll, S. G., and Surgenor, D. M.: Use of Biomechanical Equipment for the Long-Term Preservation of Erythrocytes, J. A. M. A. **168**: 404-408, 1958.
11. Keys, A., Hall, F. G., and Barron, E. S. G.: Position of the Oxygen Dissociation Curve of Human Blood at High Altitude, Am. J. Physiol. **115**: 292-307, 1936.
12. Lovelock, J. E.: Mechanism of Protective Action of Glycerol Against Haemolysis by Freezing and Thawing, Biochim. et biophys. acta **11**: 28-36, 1953.
13. Merryman, H. T.: Ice Crystal Formation in Frozen Tissues. Lecture and Review Series 53-3 Naval Medical Research Institute, 1955, March 27, pp. 1-44.
14. Mollison, P. L., Sloviter, H. A., and Chaplin, H., Jr.: Survival of Transfused Red Cells Previously Stored for Long Periods in Frozen State, Lancet **2**: 501-505, 1952.
15. Morse, M., Cassels, D. E., and Holder, M.: Position of Oxygen Dissociation Curve of Blood in Normal Children and Adults, J. Clin. Invest. **29**: 1091-1097, 1950.
16. Nahas, G. G.: Spectrophotometric Determination of Hemoglobin and Oxyhemoglobin in Whole Hemolyzed Blood, Science **113**: 723-725, 1951.
17. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry. Volume II: Methods, Baltimore, 1932, Williams & Wilkins Co.
18. O'Brien, T. G., Haynes, L. L., Hering, A. C., Tullis, J. L., and Watkins, E., Jr.: The Use of Glycerolized Frozen Blood in Vascular Surgery and Extracorporeal Circulation, Surgery (in press).
19. O'Brien, T. G., and Watkins, E., Jr.: Prolonged Heparin Activity in Stored Blood Processed by the Glycerolization-Freezing Method. (To be published.)
20. Scholander, P. F.: Analyzer for Accurate Estimation of Respiratory Gases in One-Half Cubic Centimeter Samples, J. Biol. Chem. **167**: 235-250, 1947.
21. Sloviter, H. A.: In Vivo Survival of Rabbit's Red Cells Recovered After Freezing, Lancet **1**: 1350-1351, 1951.
22. Sproul, M. T., and Sloane, M. H.: The Present Status of Research on Red Blood Cell Preservation and Plasma Sterilization in the United States. P. H. Andresen, papers in dedication of his sixtieth birthday, Copenhagen, 1957, Munksgaard, pp. 170-188.
23. Stainsby, W. N., Fales, J. T., and Lilienthal, J. L., Jr.: Rapid Spectrophotometric Method for Determining Oxygen Content of Dog's Blood, J. Appl. Physiol. **7**: 577-579, 1955.

24. Tullis, J. L., Ketchel, M. M., Pyle, H. M., Pennell, R. B., Gibson, J. G., II, Tinch, R. J., and Driscoll, S. G.: Studies on the In Vivo Survival of Glycerolized and Frozen Human Red Blood Cells, J. A. M. A. **168**: 399-404, 1958.
25. Tullis, J. L., and others: New Principle of Closed System Centrifugation, Science **124**: 792-797, 1956.
26. Valtis, D. J., and Kennedy, A. C.: Causes and Prevention of Defective Function of Stored Red Blood Cells After Transfusion, Glasgow M. J. **34**: 521-543, 1953.
27. Valtis, D. J., and Kennedy, A. C.: Defective Gas-Transport Function of Stored Red Blood-Cells, Lancet **1**: 119-125, 1954.
28. Watkins, E., Jr., Hering, A. C., and Adams, H. D.: Design and Use of a Pump-Oxygenator, S. Clin. North America **40**: 609-632, 1960.

(For Discussion, see page 631)

THE RELATIONSHIP OF THE SYMPATHO-ADRENAL SYSTEM TO POTASSIUM FLUX AND CARDIAC IRRITABILITY INDUCED BY ALTERATIONS IN BLOOD pH

Bernard Goott, M.D. (by invitation), J. C. Rosenberg, M.D. (by invitation), Richard C. Lillehei, M.D., and Fletcher A. Miller, M.D. (by invitation), Minneapolis, Minn.

PREVIOUS experiments in this laboratory have indicated that rapid reversal of the arterial pH to normal after prolonged inhalation of carbon dioxide has a far more deleterious effect upon cardiac function in the dog than the antecedent acidosis.[1, 2] Arrhythmias thus invoked frequently culminate in ventricular fibrillation, a sequence of events which may have a counterpart in clinical cases of unexplained cardiac arrest.[1, 4] Data will be presented herein demonstrating that during this critical period a rapid loss of myocardial potassium and a significant diminution of plasma levels of circulating catecholamines occur.

During hypercapnia there is a gradual rise in the levels of serum potassium, phosphorus, and glucose with a concomitant drop in the level of ultrafiltrable calcium. In the immediate post-hypercapnic period, instead of the normal relationship of these ions being restored, there is a further divergence, the ratio of calcium to potassium dropping to one third of the control.[3] This critical period has a temporal relationship to the onset of arrhythmias and fibrillation, which may, however, be prevented by measures designed to maintain a normal ratio of ion concentrations.[5]

The knowledge that levels of catecholamine in plasma are elevated during respiratory acidosis, along with our previous demonstration that epinephrine administration prevented post-hypercapnic ventricular fibrillation,[6] suggested to us that the rapid changes in ion concentration when the blood pH is suddenly advanced to normal might be associated with a drop in circulating catecholamine levels. This stimulated further investigation concerning the relationship of the sympatho-adrenal system to the fluctuations in ion concentration that occur with changes in pH and/or carbon dioxide tension.

From the Department of Surgery, University of Minnesota, and Mount Sinai Hospital Jay Phillips Research Laboratory, Minneapolis, Minn.

This study was supported by grants from the Hartford Foundation and the Minnesota Heart Association.

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

METHOD

The following groups of experiments were carried out on healthy mongrel dogs.

Group I.—In 7 dogs, simultaneous sampling of potassium levels in arterial and coronary sinus blood were performed during respiratory acidosis and in the critical post-hypercapnic period, plus analysis of plasma epinephrine and norepinephrine during the same period.

Group II.—In 14 dogs, measurements of potassium were carried out as in Group I, except that these animals were treated with either epinephrine or norepinephrine in the immediate post-hypercapnic period.

Group III.—In this experiment, arterial and coronary sinus potassium levels were measured in 13 normocapnic dogs following rapid intravenous administration of epinephrine or norepinephrine in a dosage sufficient to produce arrhythmias (8 micrograms per kilogram of body weight).

Group IV.—In this series of 8 dogs, the experiments were similar to Group III, save that the liver was excluded from the circulation by clamping the hepatic artery and portal vein prior to administration of the catecholamines.

Group V.—This consisted of the measurement of arterial potassium levels during the inhalation of 30 per cent carbon dioxide in oxygen in a series of 8 adrenalectomized dogs.

The standard experimental technique of inducing ventricular fibrillation following respiratory acidosis, as described previously, was employed.[2] In addition, in each animal a cardiac catheter was inserted into the coronary sinus via the jugular vein under fluoroscopic control. The position of the catheter was subsequently checked at autopsy. Simultaneous samples of arterial and coronary sinus blood were obtained at appropriate intervals for potassium analysis. Electrocardiographic and blood pressure tracings were recorded intermittently.

Plasma catecholamines were measured by the technique of Weil-Malherbe and Bone.[7]

TABLE I. PLASMA CATECHOLAMINE LEVELS DURING INHALATION OF CARBON DIOXIDE AND IN THE IMMEDIATE POST-HYPERCAPNIC PERIOD

		ACIDOSIS			POST-ACIDOSIS	
	CONTROL	15 MIN. CO_2(30%)	2 HOURS CO_2(30%)	4 HOURS CO_2(40%)	3 MIN.	8 MIN.
Epinephrine (microgram/L.) (mean)	0.29	1.51	2.06	5.44	1.35	3.20
Standard error	±0.1	±0.53	±0.38	±1.06	±0.45	±0.98
Norepinephrine (microgram/L.) (mean)	2.09	4.41	5.08	9.40	5.80	5.68
Standard error	±0.2	±0.62	±0.55	±1.01	±0.95	±0.97
Blood pH (mean)				6.73	7.39	
Standard error				±0.01	±0.03	

RESULTS

Group I.—The results in this group are summarized in Fig. 1. It can be seen that, as the dog inhales carbon dioxide, the potassium level gradually rises in both the arterial and coronary sinus blood, but there is no significant difference between these two sites. After 4 hours on carbon dioxide the pH has dropped to 6.7 and the potassium levels in the arterial and coronary sinus samples are almost identical. In the immediate post-hypercapnic period there is a definite rapid rise of the potassium in both systems; however, the coronary sinus samples were consistently higher by an average of 1.5 mEq. per liter.

The epinephrine and norepinephrine titers during this same period are summarized in Table I. A gradual rise in both of these substances occurs throughout the period of acidosis, particularly when the animal inhales 40 per cent carbon dioxide. This is in complete agreement with data previously reported by Nahas.[8] In the post-hypercapnic period, at the identical time when the potassium divergence described in Fig. 1 is maximal, these levels dropped precipitously.

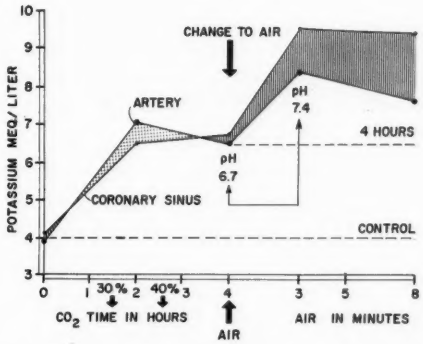


Fig. 1.—This graph illustrates changes occurring in the levels of potassium in arterial and coronary sinus blood during respiratory acidosis and in the immediate post-hypercapnic period in a series of 7 dogs. The negative arteriovenous difference in the post-hypercapnic period occurred consistently in all of the experiments. The difference ranged from −0.7 to −2.7 mEq./L. at 3 min. and from −0.7 to −3.0 mEq./L. at 8 min.

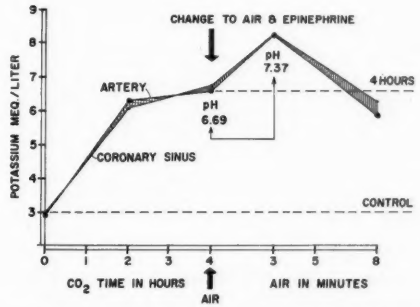


Fig. 2.—This graph illustrates the average effect on potassium levels in a series of 7 experiments when epinephrine is administered in the post-hypercapnic period. The virtual elimination of the negative arteriovenous difference that occurs in control dogs during this period was a consistent finding in all these experiments. The difference ranged from −0.5 to +3.5 mEq./L. at 3 min. and from −1.3 to +0.5 mEq./L. at 8 min. Similar results were obtained in a series of 7 dogs when norepinephrine was administered during this period.

Group II.—When epinephrine or norepinephrine is administered in the post-hypercapnic period continuously intravenously, at a rate sufficient to maintain (adequately) the systemic blood pressure, the serum potassium rises but divergence of the potassium levels does not occur; this is demonstrated in Fig. 2. The arrhythmias seen in this period are more typical of an epinephrine effect and generally rapidly revert to normal.

Group III.—Fig. 3 illustrates the changes that occur in the levels of arterial and coronary sinus potassium when either epinephrine or norepinephrine in a dose of 8 micrograms per kilogram is rapidly administered to normocapnic dogs. There is a biphasic effect with arterial potassium rising more rapidly than that of the coronary sinus to reach peak levels at 1 to 1½ minutes and then dropping gradually below the coronary sinus levels. It is of interest that, although the peak in arterial potassium occurs at 60 to 90 seconds, the greatest arteriovenous difference occurs earlier and is associated with severe arrhythmias.

Group IV.—Exclusion of the liver from the circulation by the method described does not prevent the arrhythmias following infusion of epinephrine or norepinephrine despite the fact that there is no rise in potassium levels or any major arteriovenous difference, as illustrated in Figs. 4 and 5.

Group V.—Adrenalectomy did not prevent the arterial potassium rise in dogs breathing 30 per cent carbon dioxide for 1 hour. The dogs were intolerant of higher carbon dioxide levels or more prolonged periods of exposure.

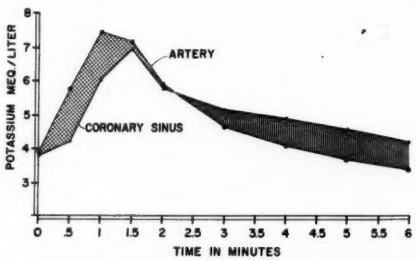


Fig. 3.—This graph illustrates the average changes that occur in the levels of potassium in arterial and coronary sinus blood when epinephrine was administered to a series of 13 normocapnic dogs in a dosage of 8 micrograms per kilogram of body weight. This biphasic effect was a consistent finding and could also be produced by administration of norepinephrine. The arteriovenous difference was usually greatest at 30 seconds and ranged from +0.7 to +3.5 mEq./L. for epinephrine and from +0.5 to +2.0 mEq./L. for norepinephrine.

DISCUSSION

Dogs inhaling high concentrations of carbon dioxide exhibit a gradual, although significant, sustained rise in blood levels of potassium, epinephrine, and norepinephrine. Tenney[9] has suggested that the serum potassium elevation is the result of release from the liver following sympatho-adrenal stimulation. However, since we were able to demonstrate an elevation of the serum potassium in the adrenalectomized animals, it must be presumed that this hepatic response can be triggered by stimuli other than the catecholamine release from the adrenal glands.

Fenn[10] postulates that, with the inhalation of high concentrations of carbon dioxide, large quantities of potassium are released from the liver, sufficient to raise serum levels over and above a shift of potassium into the tissues, which he believes occurs under these conditions. The absence of any significant arteriovenous divergence during the inhalation of carbon dioxide, as shown in Figs. 1 and 2, should not necessarily be construed as indicating lack of myocardial

potassium uptake, for Brown[11] has recorded markedly increased rates of coronary flow during this period; such an increase in flow could diminish the potassium differential below the range of analytical accuracy.

The post-hypercapnic period is characterized by a negative arteriovenous difference in potassium levels. This represents a rapid directional flux of

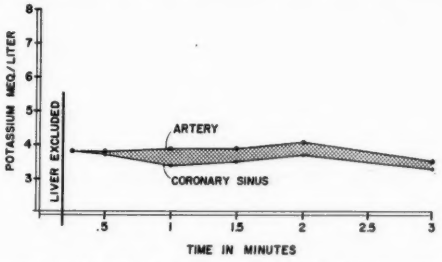


Fig. 4.—This graph illustrates the effects of epinephrine on arterial and coronary sinus potassium after exclusion of the liver from the circulation. Dosage 8 micrograms per kilogram. Average of eight experiments. Arterial potassium does not rise and the arteriovenous difference remains within the range of analytical error and ranges from +0.2 to +0.8 mEq./L.

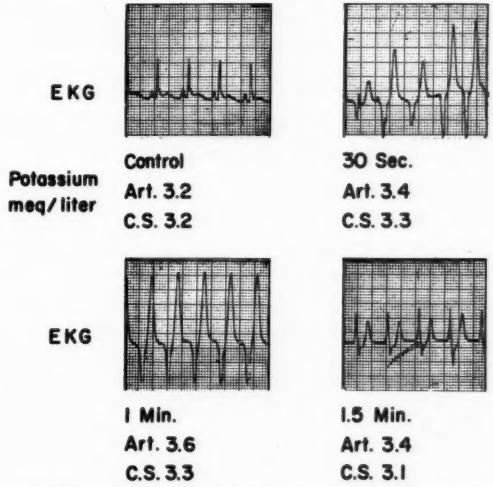


Fig. 5.—This illustration shows the effect upon the electrocardiogram of epinephrine (16 micrograms per kilogram of body weight) after exclusion of the liver from the circulation. Arterial and coronary sinus potassium levels are shown.

potassium from the myocardium immediately prior to the development of ventricular fibrillation. In addition, a precipitous fall in levels of catecholamine occurs during this critical phase.

Since epinephrine or norepinephrine administration during the critical post-hypercapnic period protects against ventricular fibrillation, it is of added significance that these substances, as shown in Fig. 2, will also prevent the rapid loss of myocardial potassium that normally occurs in the posthypercapnic period. In fact, in some dogs, administration of these drugs effected a reversal

to a positive difference. In normocapnic dogs, epinephrine and norepinephrine regularly caused a positive arteriovenous difference as shown in Fig. 3, indicating an uptake of potassium by the heart. We were not able to demonstrate such uptake of potassium after exclusion of the liver from the circulation.

In conclusion we are advancing the hypothesis that the development of ventricular fibrillation in the post-hypercapnic phase may be due to a very rapid loss of potassium from the myocardium incident to a drop in plasma catecholamine levels, and that epinephrine or norepinephrine protects the heart under such conditions by preventing this rapid potassium flux. Our data indicate that the rapidity of loss of myocardial potassium is more important in initiating the fibrillation than the mere rise of serum potassium in the circulating blood.

SUMMARY

1. During respiratory acidosis there is a rise in the potassium levels in both arterial and coronary sinus blood. There is no significant difference in these levels while acidosis is maintained but, in the immediate post-hypercapnic period, there is a further rapid rise in these levels, that of the coronary sinus being much higher which indicates loss of myocardial potassium. This period of significant arteriovenous difference has a temporal relationship to severe cardiac arrhythmias.

2. Plasma levels of epinephrine and norepinephrine are elevated during respiratory acidosis. The post-hypercapnic period is associated with a precipitous drop in the previously high levels. This too has a temporal relationship to the arrhythmias occurring at this time.

3. The administration of epinephrine or norepinephrine to normocapnic dogs has a biphasic effect on myocardial potassium flux.

4. Exclusion of the liver from the circulation prevents the rise in levels of potassium that result from the administration of epinephrine or norepinephrine, but does not necessarily prevent arrhythmias. Significant arteriovenous differences in potassium levels were not detected by the method employed.

5. The administration of epinephrine or norepinephrine in the post-hypercapnic period eliminates the negative arteriovenous difference in levels of potassium and prevents the occurrence of ventricular fibrillation.

REFERENCES

1. Miller, F. A., Brown, E. B., Buckley, J. J., Van Bergen, F. H., and Varco, R. L.: Respiratory Acidosis: Its Relationship to Cardiac Function and Other Physiologic Mechanisms, Surgery **32**: 171-183, 1952.
2. Brown, E. B., Jr., and Miller, F. A.: Ventricular Fibrillation Following a Rapid Fall in Alveolar CO_2 Concentration, Am. J. Physiol. **169**: 56-60, 1952.
3. Brown, E. B., Jr., and Prasad, A. S.: Plasma Ultrafiltrable Calcium Concentration in Posthypercapnic Ventricular Fibrillation, Am. J. Physiol. **190**: 462-466, 1957.
4. Greene, N. M.: Fatal Cardiovascular and Respiratory Failure Associated With Tracheotomy, New England J. Med. **261**: 846, 1959.
5. Goott, B., and Miller, F. A.: Prevention of Posthypercapnic Ventricular Fibrillation in Dogs, J. THORACIC SURG. **38**: 630-638, 1959.

6. Miller, F. A.: Ph.D. Thesis. Studies in Respiratory Acidosis, The Graduate School, University of Minnesota, December, 1955, pp. 143-145.
7. Weil-Malherbe, H., and Bone, A. D.: Adrenergic Amines in Human Blood, Lancet 1: 974-977, 1953.
8. Nahas, G. G., and Ligou, J. C.: Influence du pH et des catecholamines sur la consommation en oxygene du chien. Des comptes rendus des seances de l'Academie des Sciences, t. 248, p. 2132-2134, April 6, 1959.
9. Tenney, S. M.: Sympatho-adrenal Stimulation by Carbon Dioxide and the Inhibitory Effect of Carbonic Acid on Epinephrine Response, Am. J. Physiol. 187: 341-346, 1956.
10. Fenn, O., and Tomoaki, A.: Effects of Carbon Dioxide Inhalation on Potassium Liberation From the Liver, Am. J. Physiol. 185: 567-576, 1956.
11. Brown, E. B., Jr., and Mowlem, A.: Potassium Loss From the Heart During the Immediate Posthypercapnic Period, Am. J. Physiol. (To be published).

DISCUSSION

(PAPERS BY ROE [PAGE 561], MUNOZ [PAGE 568], MCCAUGHAN [PAGE 574], MEREDITH [PAGE 582], MOULDER [PAGE 588], MCLAUGHLIN [PAGE 602], O'BRIEN [PAGE 611], GOOTT [PAGE 625], AND THEIR ASSOCIATES)

DR. FREDERICK S. CROSS, Cleveland, Ohio.—In the process of developing a subcoronary aortic valve prosthesis, Drs. Gerein, Richard Jones and I have gone off on the tangent of developing a single leaflet type prosthesis. Certainly, there are clinical instances when this will be entirely satisfactory, if not preferable to replacing the entire aortic valve.

Our work has been divided into the proper design of the leaflet, and techniques of insertion. The leaflet that we have been using is constructed over a cast of the sinus of Valsalva area, utilizing particularly the noncoronary cusp. It consists of a backing of polyurethane, to which is sutured, at the present time, pericardium either as a single or double layer. The leaflet is rather easily inserted through a longitudinal incision. A single suture is placed to anchor the apex with an instrument remarkably similar to the one described by Dr. Roe. A layer of continuous sutures is then placed across the top edge of the backing.

[Slide] From this slide showing the valve in systole, it can be seen that the prosthetic pericardial leaflet is remarkably similar in the way it "floats" open to the normal valves. In reviewing the movies of valve function, it is difficult to distinguish the prosthetic cusp from the normal ones if one does not see the suture line shown here.

[Slide] This slide shows the leaflet to be perfectly competent during diastole. The competency of the valve after placement of the prosthetic monocusp has been checked with aortic pressure tracings.

[Slide] Unfortunately, the pericardium does not hold up well, and there is an early deposit of fibrin at the point of its contact with the other valve leaflets, which in a period of about 60 hours ends up looking like this.

Now, as I said, although the materials that we have are not entirely satisfactory, I think the design of the leaflet is good, and the future of this work lies in the utilization of other natural tissues, such as segments of vein, rather than pericardium, and in the development of complete plastic leaflets and entire aortic valves utilizing this basic design.

DR. CHARLES A. HUFNAGEL, Washington, D. C.—Dr. Potts once said that if a man once has an idea that he thinks is really new, that he will love it like a son forever, even though it turns out to be a moron. (laughter) I hope this is not necessarily true, in that we have always continued our interest in aortic valve replacement, and our interest has been in doing this in the normal aortic position. We have accomplished aortic valve replacement both in terms of single cusp replacement, multiple cusp replacement, and single unit valve replacement.

[Slide] With the advent of new approaches to the aortic diseases we have developed the method of cardioplegia produced by local cold alone. We have now almost 100 patients

with periods of cardiac arrest ranging from 30 minutes to an hour and 40 minutes with no instance of difficulty—major difficulty—in resuscitation. Most of these patients have had some form of aortic valve surgery, and approximately 50 have had replacement of either one or more cusps.

The mortality rate in this group has been approximately 28 per cent over-all, and in the last 20 patients it has been less than half of this figure. None of these fatalities has been due to failure of the heart to resume a satisfactory beat.

This shows one of the types of cusp which is currently in use and which seems to be highly adaptable.

[Slide] This is a valve composed of a Dacron cusp which is impregnated with silicone rubber. The self-sealing feature of this cusp necessitates only a few sutures at the base, and healing occurs into this edge of the self-seal which is cloth alone. Tissue does not grow into this but into the leaflet itself, and this preserves the elasticity of the leaflet.

[Slide] It has been our impression experimentally that when tissue grows into a cloth leaflet, it stiffens, and we have had to reoperate upon 1 patient because of this stiffening. This is the cloth, impregnated with rubber.

[Slide] This shows another view of the same thing.

[Slide] This is a more sophisticated version of this for the noncoronary cusp with a stape in the cusp edges. This makes fixation extremely rapid. We have usually placed the cusps in the coronary cusp by using sutures as Dr. Roe described in his beautiful presentation of this total valve replacement.

It seemed to us that the annulus of the aorta is still a scalloped curve, and we strongly believe that the base of the artificial valve or cusp must also follow this curve. This area is the true annulus which is tremendously tough, and which holds sutures well. When one tries to follow a constant single plan, one must either enter myocardial fiber or aorta, and the tissue adjacent to the annulus of the aorta is soft.

[Slide] This shows a further adaptation on account of the sealing. The three cusps are a single unit, and this also utilizes the single principle of what we have termed a self-seal, which minimizes the use of a housing. This is a highly functional total valve replacement with which we are highly gratified.

DR. ROBERT W. OLWINE, Boston, Mass.—While considering possible methods for extraction of calcium deposits from diseased heart valves, Dr. Watkins and I thought of applying the principle of the Stryker cast cutter in shattering rigid plaster without disturbance of soft dressings and tissue. We found that a 26,500 cycle ultrasonic sound probe applied directly to autopsy specimens of calcified valves efficiently shattered and extracted the calcified concretions.

We thought, however, it was necessary to determine exactly what damage might occur to adjacent soft tissue. The ultrasound probe was applied directly to the septal leaflet of a number of normal dog tricuspid valves. Immediately after application of the ultrasound probe, petechiae were seen on the valve cusp. This specimen shows a valve that was treated approximately 35 days prior to sacrifice, demonstrating an organized hematoma on the valve cusp. The cusp is becoming sclerotic.

This slide shows a lesion that is approximately 86 days old, demonstrating considerable sclerosis and thickening of the valve cusp.

This is another specimen at 77 days [slide] and shows essentially the same changes.

The essential histologic pattern is that of a rather bizarre, loose, fibrous structure somewhat myxomatous in nature within the valve cusp substance.

It is also interesting to note that there is no apparent damage occurring to the conduction system or to the aortic valve directly opposite the point of ultrasound application.

We would like to emphasize that there are certain dangers associated with some of the techniques that are being used for extraction of calcium concretions from valves. We might have, in ultrasonic sound, a useful tool for the experimental production of valvular lesions permitting subsequent study of the hemodynamic changes occurring in hearts which have had a chronic lesion closely resembling that of rheumatic heart disease.

DR. DON R. MILLER, Kansas City, Kans.—In Dr. Allbritten's absence I would like to bring attention to the blood phase which, in addition to the gas permeability of the membranes, appears to be important in the oxygen uptake by blood in membrane oxygenators.

[Slide] We have been working in the laboratory using a membrane device of basic design similar to the Clowes oxygenator, but which utilizes simplified supported Silastic membrane bags in which are incorporated features to induce mixing within blood films and to control thickness of blood films. One of the bags is shown in this slide.

Basic studies by Thomas Marx of the Midwest Research Institute, designer of this oxygenator, indicate there is laminar flow in conventional models of membrane oxygenators and a static layer of blood [slide], which is depicted rather simply here, adheres to the surface of the membrane and acts as a barrier to the uptake of oxygen by the blood not in immediate contact with the membrane.

[Slide] The advancing interface between saturated and unsaturated blood has been photographed in this static blood film experiment in which blood in a diffusion cell separated from oxygen by this membrane was observed through a Lucite plate. This static layer increases the distance through which oxygen must diffuse before reaching the unsaturated blood. The introduction of internal flow obstructions to produce turbulence within the blood film has resulted in greater oxygen uptake, even with thicker blood films than those used in the laminar flow experiments.

Other changes which theoretically should increase oxygen uptake in the blood phase, but which are more difficult to accomplish in a membrane oxygenator, are greater velocity to decrease the thickness of the static layer and thinner blood films to decrease the length of the oxygen diffusion path.

DR. PAUL F. WARE, Worcester, Mass.—I would like to present very briefly, if I may, one other method of getting at this problem, particularly for those of us who perhaps are only occasional members of perfusion groups and must use every parameter we possibly can for safe monitoring.

[Slide] We have been using in the laboratory and also clinically a type of reflection oximeter developed by Dr. M. Polanyi of the American Optical Company and tested by Hehir in the chemistry laboratories of the St. Vincent Hospital.

Now, it's very difficult to use a spectrophotometric method, because we do destroy samples. The method is lengthy, and it doesn't give us the information we need in the course of perfusion. Originally, Zijlstra did produce a reflection oximeter, and this was further developed by Rodrigo. They used, however, only one wave length, and this type has had very little application clinically.

In our type of oximeter two wave lengths are utilized. It is an absolute oximeter. You can use it with either dog blood or human blood, and you do get a practically instantaneous recording. Other virtues are that it is independent of hemoglobin content, oxygen saturation, or temperature.

[Slide] Just briefly, this is a simple tungsten lamp at this point, cuvette, and blood sample. There is an angle of 135 degrees here, and the density of the blood is reflected back. There are interference filters here which make the blood density concentrate in a very narrow wave length back on the photo cell.

[Slide] Utilizing these two wave lengths, we then can prove that the light, diffusely reflected by an unhemolyzed sample, is correlated with the oxygen saturation. You choose

wave lengths, such as has been done here, to give a maximum difference between the extinction coefficients of oxyhemoglobin and reduced hemoglobin.

Utilizing this present instrument with these wave lengths, you don't have to worry about the intensity of the incident light. You don't have to think about the geometry of your arrangement, and the difference between individual blood samples doesn't enter into your calibration.

This slide simply shows that the ratio of light reflected by a nonhemolyzed blood sample is a linear function of the oxygen saturation.

[Slide] This is simply to show how small an arrangement in space is actually necessary. This is a standard pump-oxygenator, Olson type. One can run a small line off the arterial line—also off the venous line—run it through the oximeter; one simply pinches the tubing for a few seconds and takes a reading. This gives you an instantaneous recording of what the actual venous or arterial oxygen saturation is. We have found this very convenient for use clinically.

DR. WILLIAM H. LEE, JR., Charleston, S. C.—The departments of surgery and pharmacology at the Medical College of South Carolina also have been interested in these observations of the rebound arrhythmias which occur following periods of acidosis. I just wanted to bring up the fact that other stimuli besides hypercapnia can set off the same reaction.

[Slide] For example, in this slide, which shows the epinephrine and norepinephrine levels which occur following azygos flow—this is used to produce a situation with a hypoxic or ischemic acidosis, and during the 10-minute phase of reduced (azygos) flow, one sees a fairly marked depression in cardiac contractile force, despite a marked increase in the concentration of norepinephrine and epinephrine.

In this instance, following the azygos flow period, there is a rather marked rebound. In this particular group of experiments, we found that there is a direct relationship between the contractile force of the heart and the concentration of these two substances when the pH is sufficiently high to allow the heart to respond to this stimulation—that is, in the range of 7.3 or above.

[Slide] This is a similar experiment which shows the azygos flow preparation, again with marked rebound in the middle tracing (contractile force). Sometimes the catecholamine levels may be one hundred times the control levels.

[Slide] The same situation occurs here—again a rebound—except that it is produced by an unusually low flow rate during cardiac bypass (20 c.c./Kg./min.) rather than by azygos flow.

[Slide] Concerning the infusion of epinephrine, this slide demonstrates the inability of the heart to respond to arterenol stimulation during acidosis, whether it be hypercapnic or metabolic, produced by ischemic flow rates. The arterial pH during azygos flow is noted to be high. After the venae cavae are released, there is a marked rebound in cardiac contractile force and a fall in arterial pH with an associated decrease in responsiveness to arterenol stimulation. As the cardiac output and tissue perfusion returns to normal, the pH progressively rises and the response to arterenol injection returns to control values. During this latter phase, an increased irritability of the heart has been observed.

This slide shows the effect of rapid arterenol infusion. The infusion of arterenol, in itself, may produce a metabolic acidosis, as one sees the pH values begin at 7.6 and drop down to 7.26, after 105 minutes of the perfusion.

BRONCHOSCOPY AND BACTERIEMIA

Sheldon Oscar Burman, M.D. (by invitation), Pittsburgh, Pa.*
*With the technical assistance of Miss Mollie L. Hill, M.T. (A.S.C.P.)***

IN THE ROUTINE diagnosis and treatment of pulmonary disease, bronchoscopy is an essential and commonplace adjunct. Facility in the technique of bronchoscopy is one of the earliest skills acquired by the thoracic surgical resident and its use is normally attended by little immediate mortality or obvious morbidity. Nevertheless, the post-bronchoscopic course of many patients is frequently marked by the appearance of a mild fever of 1° to 3° F. which occurs about 4 hours following the procedure, lasting perhaps 24 hours, and often passing unnoticed. The etiology of this fever has heretofore been a subject of speculation and has been variously ascribed to one or more of these mechanisms: (1) retention of secretions from depression of the cough reflex and inhibition of ciliary motility due to preoperative sedation and to the anesthesia employed; (2) the implantation of pyogen-laden secretions into previously uninfected parts of the lung; (3) auto-tuberculinization, or the host's response to the foreign protein presumably liberated from traumatized organisms; and (4) autoreaction of the host to blood liberated from bronchoscopic trauma to the endobronchial mucosa.

In a tuberculosis hospital most bronchoscopies are performed just before operation, after prolonged antituberculous chemotherapy, to verify the absence of active endobronchial disease. Nearly all of these patients have clean, dry, tracheobronchial trees, with stable, nonexudative disease, and the bronchoscopy is usually easy, swift, and relatively atraumatic. Indeed, the incidence of an intrabronchial pathologic condition is so low that the need for bronchoscopy has been seriously questioned.[16] Thus, it seemed to us that the accepted and time-honored explanations for the post-bronchoscopy fevers were not pertinent to most of our patients. Because fever accompaning endoscopy by other routes, particularly urethral, is known to be related to transitory bacteriemia, we undertook to determine whether the fevers associated with bronchoscopy might have their origin in the dissemination of blood-borne bacteria.

From the Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

*Teaching Fellow in Thoracic Surgery.

**From the Glenn Dale (Tuberculosis) Hospital, Glenn Dale, Maryland.

PROCEDURE

A total of 52 patients undergoing bronchoscopy, 50 with tuberculosis and 2 with bronchogenic carcinoma, were studied as follows: careful records of the state of their disease and previous antibiotic therapy were kept. The daily temperatures for the week preceding, and on the morning of, bronchoscopy were recorded. A rectal temperature was taken upon the patient's return to the ward, 2 hours later, then every 6 hours for 2 days. During bronchoscopy the presence or absence of endobronchial inflammation, purulent secretions, and traumatic bleeding was noted. In all cases, 5 per cent topical cocaine anesthesia was used. The sputa or gastric washings of all patients except 1 were repeatedly negative on culture for tubercle bacilli before bronchoscopy but acid-fast bacilli were present in smears of 2. One patient yielded organisms both on smear and culture. The disease was radiographically stable in all cases.

After preparing the skin with 70 per cent alcohol and tincture of iodine, 15 c.c. of venous blood was drawn for culture just before, 10 minutes after, 2 hours after, and 4 hours after bronchoscopy. If a fever of 1° F. or more occurred at any time within the next 2 days, blood was drawn again for culture. Duplicate blood samples of 5 c.c. each were inoculated into sterile, 8 oz., screw-top bottles containing a trypticase-agar slant and 50 c.c. of trypticase-soy broth. Cultures were grown under aerobic and anaerobic conditions and inspected twice daily for 21 days. For cultures of the tubercle bacillus, blood was inoculated into agar, Dubos, and oleic acid media (two parts blood and eight parts medium) and inspected twice daily for 10 days. Where growth was noted, transplantation was done to thioglycolate broth, methylene-blue agar, inulin agar, mannitol broth, and 7.5 per cent sodium chloride brain-heart infusion broth. If, at the end of 21 days, no growth was noted the trypticase-soy broth was centrifuged, a smear made, and stained with Gram's stain. If organisms were seen on the smear, the sediment was subcultured. At the end of 10 days, all the blood from each patient's combined samples for tubercle bacilli was pooled, digested with sodium hydroxide and hydrochloric acid, centrifuged, and smears were prepared and stained for acid-fast bacilli with carbolfuchsin stain.

Of the 52 patients studied, none was found by bronchoscopy to have purulent secretions and none was febrile before or at the time of bronchoscopy. Mild abrasion of the bronchial mucosa with a little bleeding occurred with 2 patients.

Although it is recognized that nonhemolytic staphylococci can produce serious disease, positive blood cultures containing these organisms were excluded from this series because they are common contaminants. To be acceptable, all staphylococci were required to exhibit hemolysis and to grow in a 7.5 per cent sodium chloride brain-heart infusion medium.

RESULTS

Twenty-four (46 per cent) patients had 1° to 3° F. of fever beginning 4 hours after bronchoscopy. No significant fever was detected earlier than 4

hours after the procedure. Six patients had fever lasting more than 6 hours and in 3 of these the fever persisted for 24 hours. Of the 24 patients with fever, 8 (33.3 per cent) produced positive blood cultures. No positive blood cultures were found in samples collected before bronchoscopy and no positive cultures during or after bronchoscopy were obtained from nonfebrile patients. The following bacteria were isolated from the blood: 2 patients had gamma streptococci, 3 patients had *Streptococcus viridans,* 2 patients had hemolytic *Staphylococcus albus,* and 1 patient had both *Streptococcus viridans* and *Escherichia coli.* All organisms grew under aerobic conditions with the exception of one of the gamma streptococci which was an anaerobe. All cultures yielded moderate to heavy growth. One positive blood culture was obtained during bronchoscopy, five were obtained 2 hours following bronchoscopy, and two were obtained 4 hours after the procedure. Although 6 patients had fevers lasting more than 6 hours after bronchoscopy, no positive cultures were grown from later specimens. No cultures positive for tuberculosis were found in any of the samples. It is conceivable that with larger and more frequent sampling a higher percentage of positive cultures might have been obtained.

DISCUSSION

It has been well established that bacteriemia may be induced by dental or urological manipulation.[3, 10, 15] Okell and Elliott[11] observed a transient bacteriemia (preponderantly the alpha streptococcus) following dental extraction in 72 per cent of patients with oral infections and in 32 per cent of those without obvious sepsis. Burket and Burn[2] demonstrated the dental origin of the bacteriemia by implanting a nonpathogen *(Serratia marcescens)* around the teeth and then recovering it from the blood stream following intra-oral manipulation. The same phenomenon has been observed by Elliott[5] after tonsillectomy. The incidence of bacteriemia following transurethral prostatic resection, as reported by Merritt,[8] and by Creevy and Feeney[3] ranges from 12 to 45 per cent. Cystostomy, cystoscopy, and urethral catheterization have all been responsible for inducing not only transient bacteriemia but also subacute bacterial endocarditis.[6] The causative organisms in these cases is usually the enterococcus (a variety of streptococcus), the Friedlander's bacillus (*Klebsiella pneumoniae*), *Escherichia coli,* or *Pseudomonas aeruginosa.* Bacteriemia and endocarditis have been known to follow hemorrhoidectomy, and McCoy and Mason[9] have reported it in conjunction with carcinoma of the sigmoid colon. Unterman, Milberg, and Kranis[14] have suggested an association between sigmoidoscopic examination and subsequent bacteriemia.

The mouth and pharynx notoriously abound with bacterial flora but as one advances in an aboral direction, the bacterial population diminishes sharply until by the time the bronchi and lungs are reached, as Pecora and Yegian[12] have shown, cultures are usually sterile, even in chronic lower respiratory tract disease. Expectorations and bronchial aspirates frequently represent merely the flora of the mouth and pharynx. The most common organisms in these areas are the staphylococci, streptococci, Neisseria, and coliforms, and these organisms

are precisely the cause of 95 per cent of all cases of bacterial endocarditis.[6] During normal activity, such as chewing, tooth brushing, and probably defecating, bacteria apparently enter the blood stream, having, in fact, been cultured from the blood of normal individuals,[13] with little or no apparent harm. However, bacterial endocarditis seldom occurs in a previously normal heart[6] although it can be induced experimentally in subjects with normal hearts by repeated intravenous injection of nonhemolytic streptococci.[4, 7] In patients with acquired or congenital valvular disease, on the other hand, bacteriemia is a serious threat.

The majority of patients undergoing bronchoscopy appear to suffer few immediate ill effects from it. But the onset of bacterial endocarditis is insidious and may escape clinical detection, particularly in the presence of other concomitant disease. Despite the availability of curative antibiotics, bacterial endocarditis is lethal in about 25 per cent of all cases and the threat is greatest in the older age groups. This present study suggests that prophylactic antibiotic treatment and attention to oral hygiene is indicated prior to bronchoscopy in all patients with rheumatic or congenital heart disease for these are most susceptible to bacterial endocarditis. For patients with valvular heart disease, the American Heart Association now recommends the administration of 600,000 units of aqueous penicillin and 600,000 units of procaine penicillin 30 minutes before dental extraction and it would appear reasonable to extend this practice to those patients undergoing bronchoscopy. Also, because of the increasing incidence of organisms not sensitive to penicillin, the additional use of a wide-spectrum antibiotic is suggested.

CONCLUSION AND SUMMARY

1. Approximately 50 per cent of a series of patients undergoing bronchoscopy responded with a fever of 1° to 3° F. beginning about 4 hours after the procedure.

2. Of these, 33.3 per cent were shown to have suffered a transitory bacteriemia.

3. The organisms isolated were the alpha and gamma streptococci, hemolytic *Staphylococcus albus,* and *Escherichia coli.*

4. The danger of bacteriemia in patients with congenital or rheumatic cardiovalvular disease is stressed.

5. A program of oral hygiene and antibiotic prophylaxis is urged for patients with congenital or acquired cardiovalvular disease in whom bronchoscopy is contemplated.

REFERENCES

1. Beeson, P. B., Brannon, E. S., and Warren, J. V.: Observations on Sites of Removal of Bacteria From Blood From Patients With Bacterial Endocarditis, J. Exper. Med. **81**: 9, 1945.
2. Burket, L. W., and Burn, C. G.: Bacteriemias Following Dental Extraction. Demonstration of Source of Bacteria by Means of a Non-pathogen, Serratia Marcescens, J. D. Res. **16**: 521, 1937.

3. Creevy, C. D., and Feeney, M. J.: Infection Following Transurethral Resection of Prostate Gland, Bull. Univ. Minnesota Hosp. **20**: 319, 1949.
4. Dick, G. F., and Schwartz, W. B.: Experimental Endocarditis of Dogs, Arch. Path. **42**: 159, 1946.
5. Elliott, S. D.: Bacteriemia Following Tonsillectomy, Lancet **2**: 589, 1939.
6. Friedberg, C. K.: Diseases of the Heart, Philadelphia and London, 1956, W. B. Saunders Company, chap. 34, p. 861.
7. Libman, E., and Celler, H. L.: Etiology of Subacute Infective Endocarditis, Am. J. M. Sc. **140**: 516, 1910.
8. Merritt, W. A.: Bacterial Endocarditis as Complication of Transurethral Prostatic Resection, J. Urol. **65**: 100, 1951.
9. McCoy, W. C., and Mason, J. M., III: Enterococcal Endocarditis Associated With Carcinoma of the Sigmoid; Report of a Case, J. M. A. Alabama **21**: 162, 1951.
10. Middleton, W. S., and Burke, M.: Streptococcus Viridans Endocarditis; Clinico-Pathological Analysis of Experience in Wisconsin General Hospital, Am. J. M. Sc. **198**: 301, 1939.
11. Okell, C. C., and Elliott, S. D.: Bacteriemia and Oral Sepsis With Special Reference to Etiology of Subacute Bacterial Endocarditis, Lancet **2**: 869, 1955.
12. Pecora, D. B., and Yegian, D.: Bacteriology of the Lower Respiratory Tract in Health and Chronic Diseases, New England J. Med. **258**: 74, 1958.
13. Schatten, W. E., Desprez, J. D., and Holden, W. D.: Bacteriologic Study of Portal Vein Blood in Man, A. M. A. Arch. Surg. **71**: 404, 1955.
14. Unterman, D., Milberg, M. B., and Kranis, M.: Evaluation of Blood Cultures After Sigmoidoscopy, New England J. Med. **257**: 773, 1957.
15. Weiss, H.: Relation of Portals of Entry to Subacute Bacterial Endocarditis, Arch. Int. Med. **54**: 710, 1934.
16. Wilson, N. J.: Tr. Eighteenth Conference on Chemotherapy of Tuberculosis, Remarks following discussion, p. 89. VA Department of Medicine and Surgery, Central Office, Washington, D. C.

(For Discussion, see page 699)

ALTERED HEMODYNAMICS IN THE PULMONARY CIRCULATION FOLLOWING REAERATION OF AN ATELECTATIC LUNG

Edwin T. Long, M.D. (by invitation), William E. Adams, M.D., John R. Benfield, M.D. (by invitation), Takemaru Mikouchi, M.D. (by invitation), Arthur F. Reimann, M.D. (by invitation), and Salvatore Nigro, M.D. (by invitation), Chicago, Ill.

A CURIOUS DISCREPANCY has been reported by four independent observers concerning patients who have had long-term chronic atelectasis of one lung followed by surgical intervention and reaeration of the lung. These patients have had generally satisfactory degrees of return of function but bronchospirometry studies have regularly shown a discrepancy between ventilation and oxygen uptake in the reaerated lung. Its contribution to total oxygen uptake has been significantly lower than its contribution to total ventilation.[7, 12, 16, 19] This phenomenon has not been explained in the general consensus that reaerated atelectatic lungs regain their function after reinflation.

The subject of atelectasis and the question of its reversibility have been areas of continuing interest in this laboratory.[1, 3, 11] Benfield and his associates[3] demonstrated that in dogs an average of 14 per cent of the cardiac output was shunted through chronically atelectatic left lungs. However, the shunt was eliminated by occluding the pulmonary artery to these lungs or by surgical intervention and reaeration of the lungs. Doubt was cast on the complete reversibility of chronic atelectasis by the observation that animals with a reaerated left lung did not survive contralateral pneumonectomy. The animals died with a boggy, edematous condition in the remaining reaerated lung and they exhibited a large dilated right ventricle with a passively congested liver. Another animal with a reaerated lung following chronic atelectasis was studied during balloon cardiac-catheter occlusion of the pulmonary artery to the unaffected lung. This animal developed significant pulmonary hypertension during the period of occlusion and the peripheral arterial oxygen saturation dropped markedly.

The significance of these observations was not immediately apparent. The following experiments were designed to determine the physiologic value of a reaerated chronic atelectatic lung.

From the Department of Surgery, the University of Chicago School of Medicine, Chicago, Ill., and the Suburban Cook County Tuberculosis Sanitarium, Hinsdale, Ill.

This work was supported in part by each of the following: The John A. Hartford Foundation, Inc., New York, N. Y.; the Illinois Tuberculosis Association, Springfield, Ill.; U. S. Public Health Service Grant No. 1894 (C10).

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

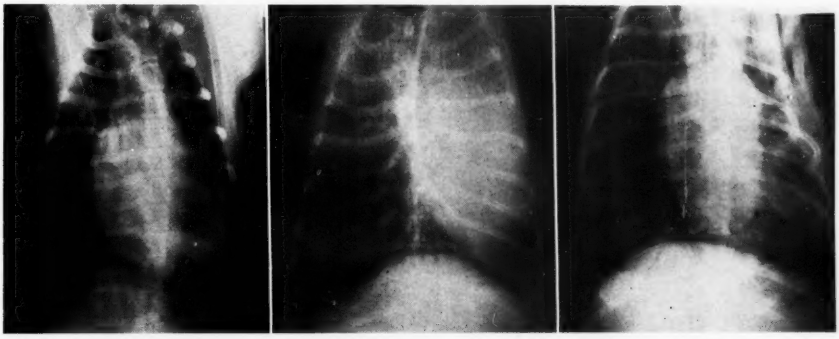
METHOD

Two groups of mongrel dogs, weighing 10 to 20 kilograms each, were used.

Group I.—12 animals. Two animals were controls.

Ten animals were subjected to chronic atelectasis of the entire left lung produced by closing the left main bronchus. This was accomplished either by bronchoscopic cautery, using 35 per cent silver nitrate solution resulting in subsequent bronchial stenosis or by surgical division of the bronchus and suture closure of the cut bronchus ends at thoracotomy.[1, 17] The atelectasis was confirmed by physical examination, bronchoscopy, fluoroscopy, and radiographic means.

After 4 to 67 weeks of atelectasis, left thoracotomy was performed with resection of the closed portions of the bronchus with end-to-end anastomosis of the opened bronchial ends, using continuous 4-0 black silk atraumatic suture material with everting technique. Before starting the bronchial anastomosis the distal bronchial tree was aspirated to remove the accumulated mucus that always filled but did not distend the bronchial system. After completing the anastomosis, the small, airless, liver-like lung was reinflated with positive pressures up to 35 mm. Hg. The lungs reaerated at lower pressures but did not resume more than 50 to 75 per cent of estimated normal size even with the highest pressures used. The reaerated lungs were pale. Postoperative chest roentgenograms were made (Fig. 1).



A. *B.* *C.*

Fig. 1.—*A*, Normal chest film.

B, Chest film which shows complete atelectasis of the left lung. The aerated lung visible above the diaphragm on the left is an enlarged accessory lobe of the right lung herniated across the midline.

C, Chest film made 4 days after left thoracotomy, bronchoplasty, and reaeration of the atelectatic lung.

Four to 120 weeks after reaeration, an evaluation of the pulmonary functions was made. Each animal was anesthetized with a continuous intravenous drip of 0.2 per cent Surital after premedication with atropine sulfate, 0.0004 Gm., and morphine sulfate, 0.015 to 0.075 Gm. Bronchospirometric ventilation studies were made using a specially constructed tracheal divider and two Benedict-Roth recording spirometers with balloon-boxes filled with either air or 26 per cent oxygen. To ensure proper position of the tracheal divider, insertion was made under fluoroscopic control using a "carina finder" as a guide (Fig. 2). After inflating the balloon on the tracheal divider to 35 mm. Hg, a test was made by hyperinflating each lung separately under fluoroscopic control. This served the dual purposes of indicating any leaks and of showing the degree to which the lungs could be expanded (Fig. 3).

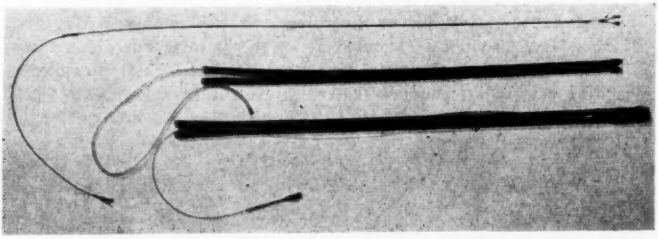
Arterial blood samples were collected from a femoral artery. Mixed venous blood samples were obtained from the pulmonary artery via ardiac catheter. Blood-oxygen content was obtained by the method of Van Slyke and Neill. Cardiac output was calculated using the direct Fick method.

Group II.—24 animals. Four animals were controls.

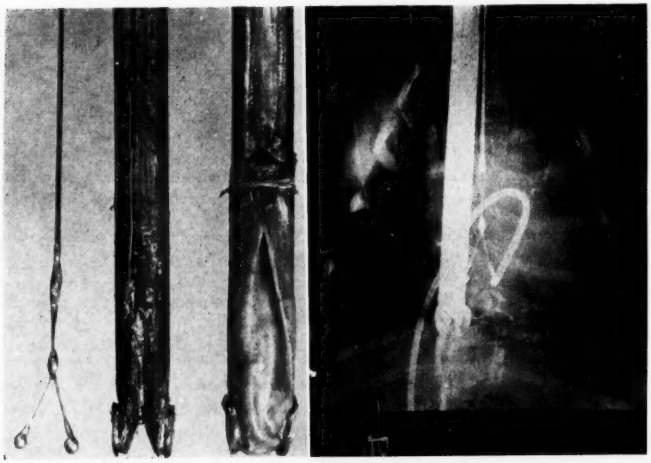
Twenty animals were subjected to chronic atelectasis of the entire left lung for 3 to 67 weeks. This was followed by surgical reaeration of the atelectatic lungs. The methods used were the same as described for Group I.

Three to 137 weeks after reaeration, the pulmonary artery pressure was studied under intravenous 0.2 per cent Surital anesthesia using a cardiac catheter, Statham P23Db strain gauge, and Grass recorder. After obtaining a satisfactory base line pulmonary artery pressure, the right pulmonary artery was temporarily occluded either by balloon cardiac-catheter obstruction (closed chest) or by cross-clamping the artery at thoracotomy (open chest). Completeness of the balloon cardiac-catheter obstruction of the right pulmonary artery was determined by cessation of oxygen uptake on that side of a bronchospirometric tracheal divider.

With the right pulmonary artery blocked, the entire cardiac output was diverted through the left reaerated lung, hemodynamically duplicating acute right pneumonectomy.



A.

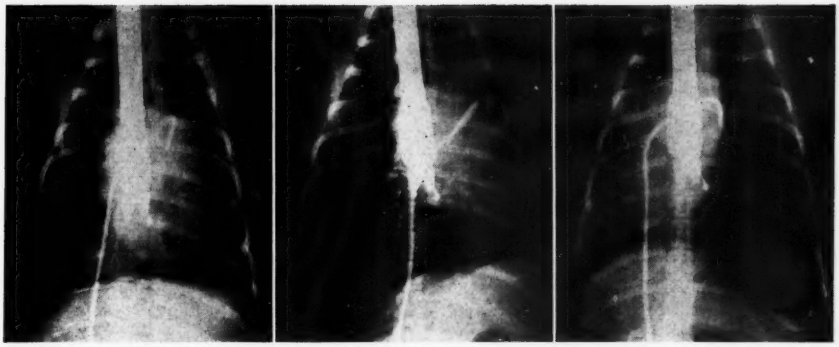


B. *C.*

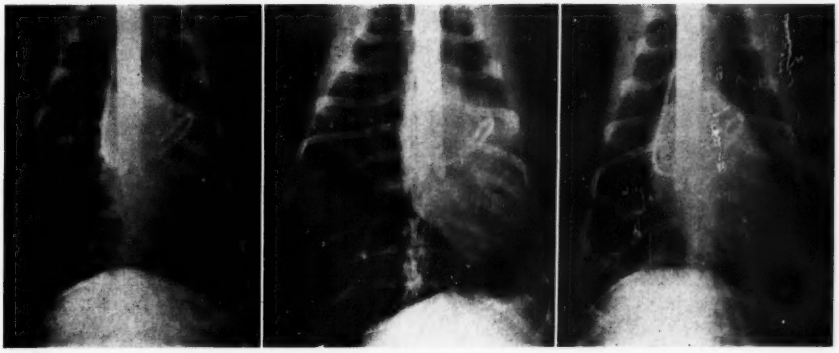
Fig. 2.—*A*, "Carina finder" and two tracheal dividers, one without balloon to show details.

B, Close-up view of the same instruments. The "carina finder" is made of piano wire and solder. The tracheal dividers are made of two large copper tubes and one small copper tube soldered together. The two large tubes have been compressed in a vise to make the cross-section of each an oval. Thus, the cross-section of the assembled divider approaches a circle.

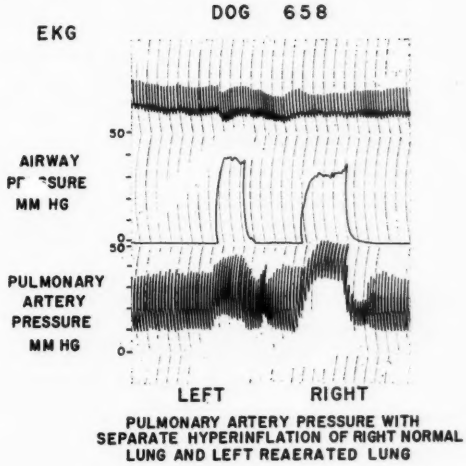
C, Chest film which shows the "carina finder" astride the carina (bronchoscopically placed) and the tracheal divider in the *wrong* position completely within the right main bronchus. When placement is correct, the crotch of the tracheal divider is coincident with the crotch of the "carina finder" fluoroscopically (see Fig. 3—*A*, *B*, *C*).



A. *B.* *C.*



D. *E.* *F.*



G. *H.*

Fig. 3.—*A*, Normal control animal with tracheal divider in place.

B, Same animal with right lung hyperinflated.

C, Same animal with left lung hyperinflated.

D, Experimental animal with reaerated left lung after 21 weeks of chronic atelectasis. Tracheal divider in place.

E, Same animal with right lung hyperinflated. Compare with *B* above.

F, Same animal with left lung hyperinflated. Compare with *C* above.

G, Same animal's pulmonary artery pressure response during hyperinflation of the left lung as shown in *F* above.

H, Same animal's pulmonary artery pressure response during hyperinflation of the right lung as shown in *E* above.

TABLE I. BRONCHOSPIROMETRY AND CARDIAC OUTPUT STUDIES (ANIM

	1†	2	3	4	5	6	7	8	9
	DOG NO.	WEEKS OF ATELECTASIS	WEEKS OF REAERATION	O_2 MIXTURE	ART. O_2 (VOL. %)	ART. O_2 SAT. (%)	VEN. O_2 (VOL. %)	VEN. O_2 SAT. (%)	A-V DIFF (VOL. %
A*	L18	0	0	26%	20.90	84	16.13	64	4.77
B	L17	0	0	Air	18.06	90	14.03	70	4.03
C	Average of 2 control animals								
1†	P28	4	40	Air	19.84	89	15.64	70	4.20
2	P3N	4	4	26%	18.33	90	15.87	78	2.40
3	P923	7	50	26%	19.85	92	14.70	67	5.15
4	P164	18	48	26%	24.38	96	19.39	76	4.99
5	P658	20	21	26%	19.25	91	15.43	74	3.82
6	P189	20	120	26%	19.79	94	15.56	74	4.23
7	P271	25	40	Air	21.30	81	17.16	65	4.15
8	P831	42	36	Air	16.10	70	12.61	55	3.50
9	P425	52	20	26%	20.02	98	16.17	79	3.85
10	P62	67	4	26%	21.44	97	16.85	76	4.59
11	Average of 10 experimental animals								

*Lines A and B contain data from control animals.
†Lines 1 through 10 contain data from the experimental animals.

Histopathologic Study.—Biopsies of the lungs of 5 of the above animals were obtained. The left lungs were biopsied in the atelectatic and reaerated states. The right lungs were biopsied only after reaeration of the left lungs. The biopsies were processed routinely by the Department of Pathology, The University of Chicago, and reviewed by Dr. Eleanor Humphreys, Professor Emeritus of Pathology.

RESULTS

There were advantages to both methods of atelectasis production. In the bronchus cautery method, atelectasis was produced without the injury of a thoracotomy and at the reaeration operation no adhesions were found. However, it was variable in its convenience and predictability if cautery was limited to a short, easily resectable length of bronchus. The method of surgical division of the bronchus was quick and easy but involved pleural trespass with a variable degree of adhesions found at the reaeration operation. There was no noticeable difference between the two methods in ease or degree of reaeration of the atelectatic lungs.

Group I.—Fluoroscopic observation of hyperinflation of the reaerated left lungs regularly demonstrated a markedly reduced compliance. All the reaerated lungs were considerably limited in their ability to expand. The loss of compliance was seemingly more significant in the upper portions of the lungs; the lower lobes expanded fairly well (Fig. 3).

The response of pulmonary artery pressure to the hyperinflation maneuver was quite revealing. When the right lung was hyperinflated, the pulmonary artery pressure elevation was proportional to the inflating pressure.[18] But when the reaerated left lung was hyperinflated, the pulmonary artery pressure was affected only slightly (Fig. 3).

The bronchospirometric pulmonary function data and the Fick cardiac output data are presented in Table I.

ES OF ANIMALS WITH REAERATED LEFT LUNGS AFTER CHRONIC ATELECTASIS

9	10	11	12	13	14	15	16	17	18
A-V DIFF. (VOL. %)	V_{O_2} RIGHT LUNG (C.C./M.)	V_{O_2} RIGHT (% OF TOTAL)	V_{O_2} LEFT LUNG (C.C./M.)	V_{O_2} LEFT (% OF TOTAL)	CARDIAC OUTPUT L./M. (FICK)	VENT. RIGHT LUNG (L./M.)	VENT. RIGHT LUNG (% OF TOTAL)	VENT. LEFT LUNG (L./M.)	VENT. LEFT LUNG (% OF TOTAL)
4.77	38.7	57	25.0	43	1.23	1.20	60	.80	40
4.03	28.1	62	17.5	38	1.13	1.13	57	.85	43
		59.5		40.5			58.5		41.5
4.20	59.0	79	15.0	21	1.76	3.9	71	1.6	29
2.40	45.0	82	10.0	18	2.28	1.5	76	.5	24
5.15	68.7	77	20.6	23	1.73	1.78	76	.55	24
4.99	75.0	100	0	0	1.50	3.3	83	.70	17
3.82	60.0	86	10.0	14	1.83	1.92	65	1.00	35
4.23	60.0	100	0	0	1.41	1.93	91	.18	9
4.15	32.0	65	17.0	35	1.18	1.03	62	.63	38
3.50	100.0	89	12.0	11	3.20	2.5	82	.55	18
3.85	52.0	100	0	0	1.35	2.2	76	.7	24
4.59	65.0	83	12.5	17	1.67	2.1	72	.8	28
		86.1		13.9			75.4		24.6

The use of 26 per cent oxygen in the spirometer-balloon box system seemed to help in keeping arterial oxygen saturation above 90 per cent during the bronchospirometry study. Of the 4 animals with arterial oxygen saturation over 93 per cent, 3 had no oxygen uptake in the left lung and the fourth had an oxygen uptake of 17 per cent of the total (Table I, columns 6 and 12, lines 4, 6, 9, 10).

The contribution to both ventilation and oxygen uptake of the left lung of the control animals was approximately 40 per cent of total. The contribution to ventilation of the reinflated left lung of the 10 experimental animals averaged 24.6 per cent of total and to oxygen uptake was only 13.9 per cent of total. There was an absolute increase in right lung oxygen uptake and an absolute decrease in left lung oxygen uptake compared to controls (Table I, columns 13 and 18, lines C and 11; Table I, columns 10 and 12).

Group II.—The pulmonary artery pressure data are presented in Tables II and III and Fig. 4.

Table II: The base line mean pressures of the control animals averaged 13.0 mm. Hg and the mean pressures with right pulmonary artery occlusion averaged 20.0 mm. Hg. The base line pulmonary artery mean pressures of the 20 experimental animals averaged 16.2 mm. Hg and, with right pulmonary artery occlusion, they averaged 36.0 mm. Hg. This occlusion value is over 200 per cent of base line mean pressures for the experimental animals and almost 300 per cent of the base line mean pressures for the control animals. The experimental animals did not tolerate the occlusion of the right pulmonary artery without distress. The distress was relieved by removing the occlusion.

Table III: There was apparently little difference in the qualitative results whether the temporary occlusion of the right pulmonary artery was produced by balloon cardiac-catheter occlusion (closed chest) or by cross-clamping at thoracotomy (open chest). Base line pulmonary artery pressures were higher in the open chest experiments.

TABLE II. PULMONARY ARTERY PRESSURE STUDIES OF ANIMALS WITH REAERATED LEFT LUNGS AFTER CHRONIC ATELECTASIS

	DOG NO.	WEEKS OF ATELECTASIS	WEEKS ELAPSED TO PRESSURE TEST	METHOD OF PA OCCLUSION	PAP RT. & LT. PA OPEN (MM./Hg.)		PAP LT. PA OCCLUDED (MM./Hg.)		PAP RT. PA OCCLUDED (MM./Hg)	
A*	C1	0	0	Balloon, closed chest	18/6	10	25/4	11	33/9	17
B	C2	0	0	Balloon, closed chest	22/15	17	28/22	24	32/27	29
C	C3	0	0	Thoracotomy, open chest	23/10	15	—	—	22/8	13
D	C4	0	0	Thoracotomy, open chest	21/4	10	—	—	33/15	21
Average of 4 control animals					13.0		17.5		20.0	
1†	P1N	3	35	Balloon, closed chest	25/5	12	—	—	38/18	26
2	P3N	4	8	Balloon, closed chest	25/7	12	27/10	13	40/12	22
3	P5N	4	13	Thoracotomy, open chest	25/17	19	—	—	50/26	34
4	P2N	4	15	Thoracotomy, open chest	24/10	15	—	—	55/27	35
5	P28	4	15	Balloon, closed chest	25/10	15	35/10	18	45/20	28
6	P923	7	55	Balloon, closed chest	28/7	15	33/10	18	35/15	24
7	P961	9	137	Balloon, closed chest	25/7	12	—	—	45/10	28
8	P10N	11	8	Balloon, closed chest	35/8	20	40/11	21	67/28	42
9	P164	18	48	Balloon, closed chest	22/5	12	33/8	18	75/25	40
10	P149	20	8	Balloon, closed chest	25/5	13	35/10	18	50/15	27
11	P189	20	120	Balloon, closed chest	22/10	14	27/12	16	40/17	25
12	P271	25	76	Thoracotomy, open chest	32/12	17	—	—	45/17	27
13	P124	26	121	Thoracotomy, open chest	27/17	20	—	—	50/25	33
14	P8N	35	6	Thoracotomy, open chest	46/22	27	—	—	100/50	72
15	P7N	36	4	Thoracotomy, open chest	33/12	20	—	—	82/40	58
16	P9N	36	4	Thoracotomy, open chest	45/20	30	—	—	75/32	48
17	P822	38	3½	Balloon, closed chest	23/2	9	—	—	83/18	49
18	P425	52	23	Balloon, closed chest	22/7	12	24/12	16	50/25	38
19	P299	61	34	Thoracotomy, open chest	23/10	15	—	—	60/15	33
20	P62	67	4	Balloon, closed chest	35/9	16	—	—	75/10	31
Average of 20 experimental animals					16.2		17.2		36.0	

*Lines A through D contain the data from control animals.
†Lines 1 through 20 contain the data from the experimental animals.

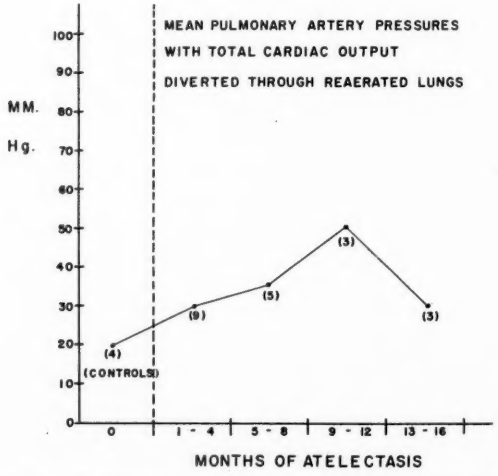


Fig. 4.—Mean pulmonary artery pressures with total cardiac output diverted through reaerated left lungs after chronic atelectasis.

TABLE III. PULMONARY ARTERY PRESSURE STUDIES OF ANIMALS WITH REAERATED LEFT LUNGS AFTER CHRONIC ATELECTASIS*

	OBSTRUCTION OF PULMONARY ARTERY BY BALLOON CARDIAC CATHETER (CLOSED CHEST)			OBSTRUCTION OF PULMONARY ARTERY BY CROSS-CLAMPING AT THORACOTOMY (OPEN CHEST)			
	BASE LINE PAP	OCCLUSION OF LEFT PA	OCCLUSION OF RIGHT PA	BASE LINE PAP	OCCLUSION OF LEFT PA	OCCLUSION OF RIGHT PA	
A	18/6 10	25/4 11	33/9 17	23/10 15	—	22/8 13	C
B	22/15 17	28/22 24	32/27 29	21/4 10	—	33/15 21	D
Average of controls	13.5	17.5	23.0	12.5		17.0	
1	25/5 12	—	28/18 26	25/17 19	—	50/26 34	3
2	25/7 12	27/10 13	40/12 22	24/10 15	—	55/27 35	4
5	25/10 15	35/10 18	45/20 28	32/12 17	—	45/17 27	12
6	28/7 15	33/10 18	35/15 24	27/17 20	—	50/25 33	13
7	25/7 12	—	45/10 28	46/22 27	—	100/50 72	14
8	35/8 20	40/11 21	67/28 42	33/12 20	—	82/40 58	15
9	22/5 12	33/8 18	75/25 40	45/20 30	—	75/32 48	16
10	25/5 13	35/10 18	50/15 27	23/10 15	—	60/15 33	19
11	22/10 14	27/12 16	40/17 25				
17	23/2 9	—	83/18 49				
18	22/7 12	24/12 16	50/25 38				
20	35/9 16	—	75/10 31				
Average of experimental animals	13.5	17.2	31.6	20.3	—	42.5	

*The data from Table III are rearranged according to the method of diversion of blood from the right lung (open chest vs. closed chest). Separate averages are shown for the two methods.

Fig. 4: The pulmonary artery pressures during right pulmonary artery occlusion tended to be higher in the animals with longer duration of atelectasis but there was considerable variation.

Histopathologic Study.—In general, the changes in the atelectatic lung were difficult to interpret because the alveolar spaces could not be distinguished. There was noticeable paucity of small blood vessels and blood-filled capillaries. Large macrophages were liberally scattered through the lung structures, both intra- and extrabronchially. The small bronchioles were collapsed and free of accumulated mucus. Only the larger bronchioles contained mucus. Hemosiderin deposits were scattered through the atelectatic tissue. Pleural thickening, while present, was minimal.

Representative photomicrographs of sections of normal and reaerated lungs are presented in Fig. 5.

The sections of reaerated lung after chronic atelectasis were revealing. The pleural surfaces were normal or minimally thickened. The subpleural zone of pulmonary parenchyma often seemed distinctly hypoinflated when compared to the remainder of the parenchyma. In this subpleural zone, the alveolar spaces were scarcely aerated whereas, in the remainder of the lung, there was overstretching of the terminal respiratory units and some disruption of alveolar walls. A number of prominent spurs or club-like processes were scattered through the lung. They were located particularly at the junction of bronchiolar arteries with perialveolar capillaries. The exact nature of these club-like processes is not known.

The sections of normal right lungs showed some enlargement of alveolar spaces and some disruption of alveolar septa.

DISCUSSION

These experimental data tend to confirm and reproduce the bronchospirometric observations in human beings of a ventilation-oxygen uptake discrepancy in reaerated chronically atelectatic lungs.

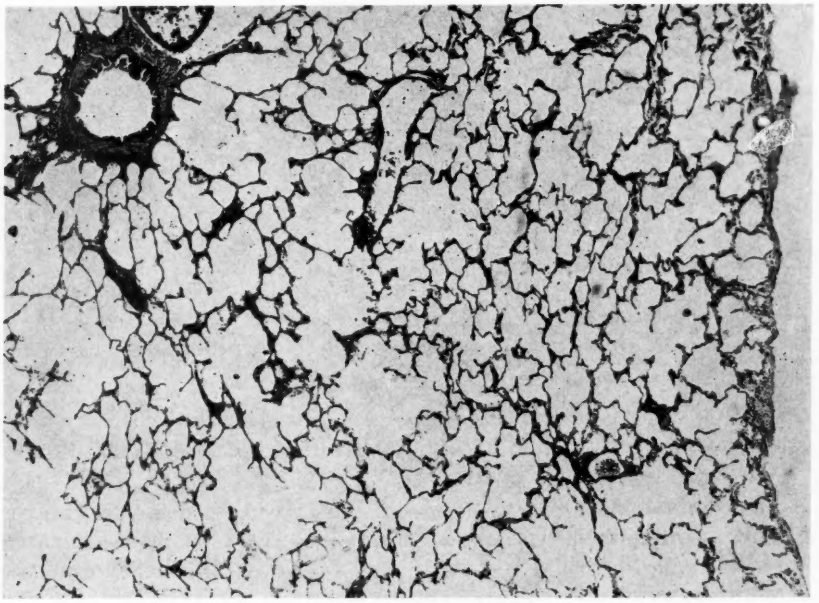


Fig. 5.—*A*, P8N. The right, unaffected lung shows some enlargement and minimal disruption of air spaces. (×56; reduced ⅙.)

Several investigators have measured the blood flow through an atelectatic left lung in the dog. They have reported a range from 0 to 40 per cent of cardiac output, with an average approximately 15 per cent.[2, 3, 4, 6, 10, 13, 14] A comparison with the present experimental observations of an average of 13.8 per cent of total oxygen uptake in the reaerated left lungs suggests that, while reaeration eliminates the shunt previously present,[3] it does not greatly improve the circulation through the lung.

This conclusion is supported by the pulmonary artery pressure data during temporary occlusion of the right pulmonary artery. It is clear that with pressures of more than 100 per cent over base line, these animals developed a considerable pulmonary hypertension. A similar right pulmonary artery occlusion in a normal animal results in less than 50 per cent elevation of base line pressure. The reaerated lungs were apparently unable to accommodate an acute

and massive increase in blood flow because of increased pulmonary vascular resistance.

While there have been few reported human bronchospirometric studies after reaeration of a chronically atelectatic lung, there have been a number of reports of routine pulmonary function tests that have shown some increase in vital capacity after recovery. This increase has usually been small, presumably due to redistribution of approximately the same intrapleural volume from one lung preoperatively to two lungs postoperatively. While it was not possible to measure vital capacity in the present experimental animals with reaerated lungs, it is clear from chest roentgenograms (see Fig. 1) that a redistribution of pleural

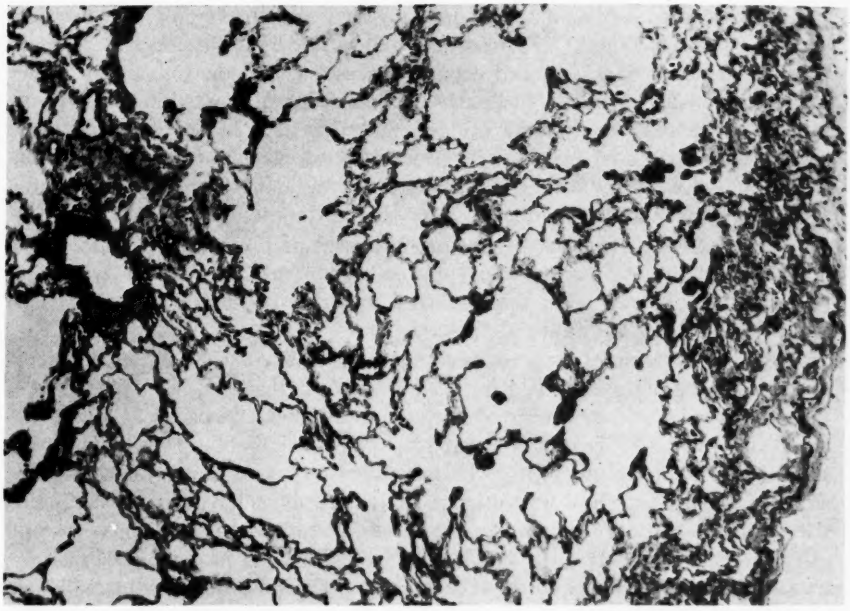


Fig. 5.—Cont'd. *B*, P10N. Eleven weeks of atelectasis and 8 weeks post-reaeration. The left, reaerated lung shows minimal thickening and infolding of the pleural surface. Note the subpleural zone of hypoinflation. There is some interalveolar septal thickening and frequent club-like formations at the junction of bronchiolar arteries with perialveolar capillaries. (×50; reduced ⅙.)

space took place when the atelectatic left lungs were reaerated. In addition, the separate hyperinflation of the reaerated lungs demonstrated that marked limitation existed in their compliance compared to the normal controls up to 2 years after reaeration. There is as yet no known reason for this reduction in compliance since previous histologic studies of atelectatic and reaerated lungs, in the absence of infection, have not shown pulmonary fibrosis or other change likely to result in decrease of pulmonary function. The diminished compliance must have been present at the reaeration operation to account for the smaller than normal size of the reaerated lungs. One observer reported that

the reaerated lungs resumed normal size several weeks or months after reaeration.[9] The present study could not confirm this observation.

An increase in pulmonary arterial resistance with decrease in flow in one lung was produced experimentally by Rosenberg[15] by wrapping one lung in a constricting bag (i.e., a marked reduction of compliance). That the mechanism of the increased resistance and decreased flow is the same in the present experiment is not certain but highly suggestive. In addition, it corresponds with Benfield's observation that contralateral pneumonectomy was not tolerated. It is known that pneumonectomy in the normal dog is followed by little pulmonary artery pressure elevation if the remaining lung is free to make compensatory adjustments. The present data suggest that contralateral pneumonectomy forced cardiac output through a smaller than normal, compensation-limited, reaerated lung.[8] The resulting pulmonary hypertension was followed by right ventricle dilatation and hepatic congestion, and the pulmonary artery pressure probably exceeded the osmotic pressure of plasma protein which results in pulmonary edema, air hunger, and death. Carlson[5] suggested that edema could be caused by altered permeability of the alveolar-capillary membrane due to hypoxia during atelectasis but it is doubtful that such altered permeability would last significantly beyond the time of reaeration.

The present histopathologic findings in reaerated lungs of subpleural hypoinflation and bronchiolar arterial spur or club formation may be wholly or partially the cause of both the increased vascular resistance and diminished compliance. The significance of these changes is not yet fully understood.

The conservation of lung tissue stands as a worthy goal in patients afflicted with chronic atelectasis of one lung. It is known that surgical reaeration of these lungs will yield a number of physiologic benefits. The present study suggests that reaeration is useful but it may fall considerably short of restoring that lung to complete normality. This statement is not an implied disagreement with the policy of surgical reaeration of atelectatic lungs but rather a suggestion that patients who have undergone reaeration operations be studied fluoroscopically, bronchospirometrically, and hemodynamically, as in the present study, whenever possible. The results of these and other follow-up studies will help to define the full clinical significance of the altered hemodynamics in the pulmonary circulation following reaeration of an atelectatic lung.

CONCLUSIONS

1. Reaeration of a chronically atelectatic lung in the dog restores its function partially. However, specific physiologic defects remain. They include a variable but persistent loss of compliance, a marked increase in pulmonary vascular resistance, a diminished pulmonary artery blood flow, and a concomitant decrease in oxygen uptake.

2. Histopathologic changes have been noted in reaerated atelectatic animal lungs. A zone of subpleural hypoinflation was noted. Bronchiolar arteries exhibited formation of spurs or club-like processes. The significance of the changes is not yet clear.

3. In human beings with a reaerated chronically atelectatic lung, a similar partial restoration of function with decrease in oxygen uptake has been reported by others.

4. From the published reports of some patients it appears that the passage of time has favorably influenced the function of reaerated atelectatic lungs. Such a favorable influence of time could not be confirmed in the present experimental study.

5. It is suggested that a complete functional evaluation of a patient with a reaerated lung after chronic atelectasis might properly include bronchospirometry and an investigation of vascular resistance and pulmonary compliance.

SUMMARY

The pulmonary functions and cardiac output of 10 dogs were studied during bronchospirometry after reaeration of a chronically atelectatic left lung.

The pulmonary arterial pressure response of 20 dogs was studied during temporary occlusion of the right pulmonary artery after reaeration of a chronically atelectatic left lung.

Normal, atelectatic, and reaerated lungs were compared histopathologically.

REFERENCES

1. Adams, W. E.: Detailed Description of a Safe and Reliable Method for Closing Large Bronchi, J. THORACIC SURG. 3: 198-200, 1933.
2. Andrus, W. de W.: Observations on the Cardio-respiratory Physiology Following the Collapse of One Lung by Bronchial Ligation, Arch. Surg. 10: 506, 1925.
3. Benfield, J. R., Long, E. T., Harrison, R. W., Perkins, J. W., Jr., Herman, G. P., and Adams, W. E.: Should a Chronic Atelectatic Lung Be Reaerated or Excised? Dis. Chest 37: 67-74, 1960.
4. Björk, V. O., and Salen, E. F.: The Blood Flow Through an Atelectatic Lung, J. THORACIC SURG. 20: 939, 1950.
5. Carlson, R. I., Classen, K. L., Gollan, F., Gobbel, W. G., Jr., Sherman, D. E., and Christensen, R. O.: Pulmonary Edema Following the Rapid Re-expansion of a Totally Collapsed Lung Due to Pneumothorax. A Clinical and Experimental Study, S. Forum 9: 367, 1958.
6. Coryllos, P. N., and Birnbaum, G. K.: The Circulation in the Compressed, Atelectatic and Pneumonic Lung, Arch. Surg. 19: 1346, 1929.
7. Forster, E.: Atelectasie pulmonaire prolongee et reversibilite functionelle, Lyon chir. 52: 484-487, 1956.
8. Gerst, P. H., Rattenborg, C., and Holaday, D.: The Effects of Reduced Pulmonary Blood Flow on Pulmonary Circulation and Respiratory Gas Exchange, S. Forum 9: 374-378, 1958.
9. Hinshaw, J. R., and Emerson, G. L.: Histologic Studies of the Lung During Recovery From Atelectasis, S. Forum 9: 390-392, 1958.
10. Keeley, J. L., and Gibson, J. G.: Experimental Atelectasis in Dogs, Surgery 11: 527, 1942.
11. Loosli, C. G., Adams, W. E., and Thornton, T. M., Jr.: The Histology of the Dog's Lung Following Experimental Collapse, Anat. Rec. 105: 697-721, 1949.
12. Mahaffey, D. E., Creech, O., Jr., and De Bakey, M. E.: Traumatic Rupture of the Left Main Bronchus Successfully Repaired Eleven Years After Injury, J. THORACIC SURG. 32: 312-331, 1956.
13. Moore, R. L.: The Volume of Blood Flow per Minute Through the Lungs Following Collapse of One Lung by Occlusion of Its Bronchus, Arch. Surg. 22: 225, 1931.
14. Peters, R. M., Loring, W. E., and Sprunt, W. H.: An Experimental Study of the Effect of Chronic Atelectasis on Pulmonary and Bronchial Blood Flow, Circulation Res. 7: 31-36, 1959.

15. Rosenberg, M. Z.: Effects of Unilateral Collapse of the Lung, Yale J. Biol. & Med. **25**: 51, 1952.
16. Samson, P. C.: Discussion of Mahaffey, Creech, and De Bakey.[12]
17. Webb, W. R., and Burford, T. H.: Studies of the Re-expanded Lung After Prolonged Atelectasis, A. M. A. Arch. Surg. **66**: 801-809, 1953.
18. Webb, W. R., and Smith, J. R.: Pulmonary Hemodynamics as Affected by Varying Levels of Respiratory Pressure, S. Forum **9**: 371-374, 1958.
19. Weisel, W., and Jake, R. J.: Anastomosis of Right Bronchus to Trachea 46 Days Following Complete Bronchial Rupture From External Injury, Ann. Surg. **137**: 220-227, 1953.

(For Discussion, see page 699)

SELECTIVE CHEMOTHERAPY OF THE LUNG DURING UNILATERAL PULMONARY ARTERIAL OCCLUSION WITH A BALLOON-TIPPED CATHETER

Nicholas P. Smyth, M.D. (by invitation), and
Brian Blades, M.D., Washington, D. C.

THE USE of pulmonary intra-arterial carcinolytic agents, administered either by trapping in the lung or by selective perfusion of the lung, has been previously reported.[1, 2] Clinical application of these techniques is hampered if a carcinoma involves the hilum of the lung, preventing access to the pulmonary artery.

Perhaps the greatest problem in selective chemotherapy of the lung is that, while the pulmonary arterial system is suitable for isolation-perfusion techniques, the great majority of pulmonary diseases, including primary bronchogenic carcinoma, apparently derive their blood supply from the bronchial arterial system.[3-12] Metastatic tumors are probably supplied by the pulmonary arterial system.[6, 12]

In the normal lung, there are both capillary and dormant precapillary communications between the two arterial systems.[11, 13-16] In the presence of primary bronchogenic carcinoma, bronchiectasis, tuberculosis, and many other diseases, extensive functioning precapillary communications develop between the two arterial systems.[3-8, 10-12] It is natural to suppose that these are left-to-right shunts.

In support of this are the findings of increased oxygen saturation of the pulmonary arterial blood in the vicinity of primary carcinoma and other diseases[5, 7, 8, 9, 17]; the failure of pulmonary angiograms to fill the pulmonary arteries in the vicinity of disease[5, 18]; and the finding of left ventricular hypertrophy in such cases.[17, 19-22]

Against this are the findings of Brofman[23] and Nemir,[24] and their associates, who used a balloon-tipped cardiac catheter to occlude the right or left pulmonary artery in the study of lung function. They found that the pressure in the pulmonary artery, distal to the occlusion, fell to pulmonary capillary levels even in the presence of extensive bronchiectasis, a disease associated with marked increase in bronchial-pulmonary arterial anastomoses. The higher

From the Department of Surgery, The George Washington University School of Medicine, Washington, D. C.

Supported in part by a research grant (CY-3777) (C-2) from the National Institutes of Health, U. S. Public Health Service.

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

pressures expected from bronchial arterial precapillary inflow were not encountered. Furthermore, the oxygen saturation of this "lacunar" blood was higher than that of the systemic blood and the pulmonary capillary blood, indicating that it was derived not from bronchial arterial inflow but, rather, from left atrial reflux; that is, blood that had passed through the pulmonary capillary bed twice.

These discrepancies are not easily reconciled, but it appears that during unilateral pulmonary arterial occlusion, perhaps as a result of some neurogenic mechanism, significant left-to-right precapillary bronchial-pulmonary arterial shunts do not occur.[23]

It seemed to us that under these conditions, it might be possible to reach both primary and metastatic tumors with a chemotherapeutic agent injected into the pulmonary artery distal to its occlusion by a balloon-tipped cardiac catheter. This technique would have the further advantages of allowing access to a pulmonary artery involved by tumor and selective, and possibly repeated, chemotherapy of the lung without thoracotomy.

The circulation and diffusion of indicator substances and the tolerance of the lung for various doses of nitrogen mustard were studied in the dog during unilateral pulmonary arterial occlusion, prior to a clinical trial of the method. Based on the observation of Creech and associates[25] that fixation or inactivation of nitrogen mustard occurs within 8 minutes in whole blood, an occlusion time of 15 minutes was arbitrarily chosen.

METHODS

1. *Circulation.*—The circulation in the lung was studied in mongrel dogs, weighing 15 to 25 kilograms, that were divided into four groups.

Group 1: A control group in which the pulmonary artery was not occluded.

Group 2: Animals in which the right pulmonary artery was occluded.

Group 3: Animals in which the right pulmonary artery and the right bronchial artery were occluded.

Group 4: Animals in which the right pulmonary artery, the right bronchial artery, and the right main-stem bronchus were occluded.

The animals were anesthetized with intravenous Pentothal sodium. Oxygen was administered by intermittent positive pressure of 14 cm. of water through a cuffed endotracheal tube. Either a No. 8½ Fr. double lumen balloon-tipped cardiac catheter* or a No. 7 Fr. single lumen cardiac catheter was inserted through the right external jugular vein and positioned in the right pulmonary artery at right thoracotomy. The pulmonary artery was occluded either by inflating the balloon or by the use of a snare (Fig. 1). In Groups 3 and 4, the bronchial arterial inflow was occluded by skeletonizing the hilum and dividing all small vessels on the isolated bronchus. In Group 4, the bronchus was occluded by a non-crushing clamp.

T-1824 (Evan's blue dye) and a saline suspension of Cr^{51} tagged red blood cells were used as indicators. To minimize errors in either method, both indicators were used simultaneously in each experiment. Indicator dilution curves were obtained in 5 animals weighing 20 to 25 kilograms in each group. Two milliliters of each indicator were slowly injected through the catheter over a 2-minute period to permit adequate diffusion in the lung. Blood samples were obtained through a Cournand needle in the femoral artery.

*Dotter-Lukas No. 1, U. S. Catheter & Instrument Corp., Glens Falls, N. Y. The standard balloon was replaced by a smaller one for animal experiments.

In the control group, samples were taken at 1, 5, 10, and 20 minutes after completion of the injection. In Groups 2, 3, and 4, the injection was started 2 minutes after pulmonary arterial occlusion, a time arbitrarily chosen to permit stabilization of the circulation. Blood samples were taken at intervals of 1 minute, 3 minutes, 5 minutes, and 10 minutes after completion of the injection. The occlusion was then released, and further samples taken 1 minute and 10 minutes later. Complete mixing was assumed to have taken place at this time and the readings for the last sample were taken to represent 100 per cent of the indicator in the systemic circulation in each experiment.

The values for T-1824 were obtained by a modification of the method of Hamilton and associates,[26] using the Beckman DU spectrophotometer. The values for Cr^{51} tagged red blood cells were obtained by a modification of the method of Spencer and associates.[27] To correct for varying radioactivity in different preparations of Cr^{51} tagged red blood cells, all readings were corrected to a uniform standard of 2.75 million counts per milliliter per 5 minutes.

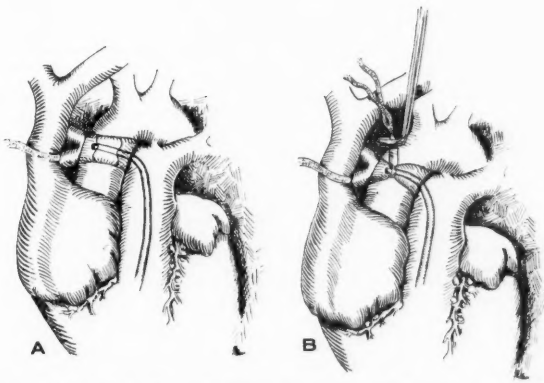


Fig. 1.—Methods used to occlude the pulmonary artery. *A*, Balloon-tipped double lumen cardiac catheter. *B*, Single lumen cardiac catheter with a snare around the pulmonary artery.

Since the behavior of inert indicators could only approximate that of a highly reactive agent like nitrogen mustard, their exact concentration in the blood was not important. We were only interested in what percentage of the total amount, if any, appeared in the systemic circulation during pulmonary arterial occlusion. A simple linear projection was therefore used in plotting the dilution curves rather than the conventional semi-logarithmetical projection, and the data were plotted as units of optical density, and counts per milliliter per 5 minutes, against time in minutes.

2. *Diffusion.*—The distribution of an indicator was also studied in 5 animals, weighing 15 to 20 kilograms, in each group. Five milliliters of methylene blue dye were injected through the catheter, and the time of appearance, the distribution, and the duration of the blue stain studied.

3. *Dose of Nitrogen Mustard.*—The tolerance of the lung for nitrogen mustard was studied in 14 animals, weighing 15 to 20 kilograms. Under intravenous Pentothal sodium anesthesia, a No. 8½ Fr. balloon-tipped double lumen cardiac catheter was inserted into the external jugular vein and positioned, in the right or left pulmonary artery, under fluoroscopic control in 9 animals and, at right thoracotomy, in 5 animals. The balloon was inflated with 50 per cent Hypaque* until the pulmonary arterial pressure distal to the balloon fell to pulmonary capillary levels. A solution of nitrogen mustard in saline (1.0 mg. per milliliter) was then injected into the blood stream distal to the balloon, in doses of 0.1, 0.2, 0.3, or 0.4 mg. per kilogram, during a period of 15 minutes of occlusion.

*Winthrop Laboratories, New York, N. Y.

On each animal, white blood cell, hematocrit, and platelet counts were obtained before the perfusion and at weekly intervals thereafter. Chest roentgenograms were obtained before and 24 hours after the perfusion, and at weekly intervals thereafter.

In a control group of 5 animals, a dose of nitrogen mustard of 0.4 mg. per kilogram was given intravenously. White blood cell, hematocrit, and platelet counts were obtained on each animal before the injection of nitrogen mustard, and at weekly intervals thereafter.

All the animals were observed for a period of 4 weeks.

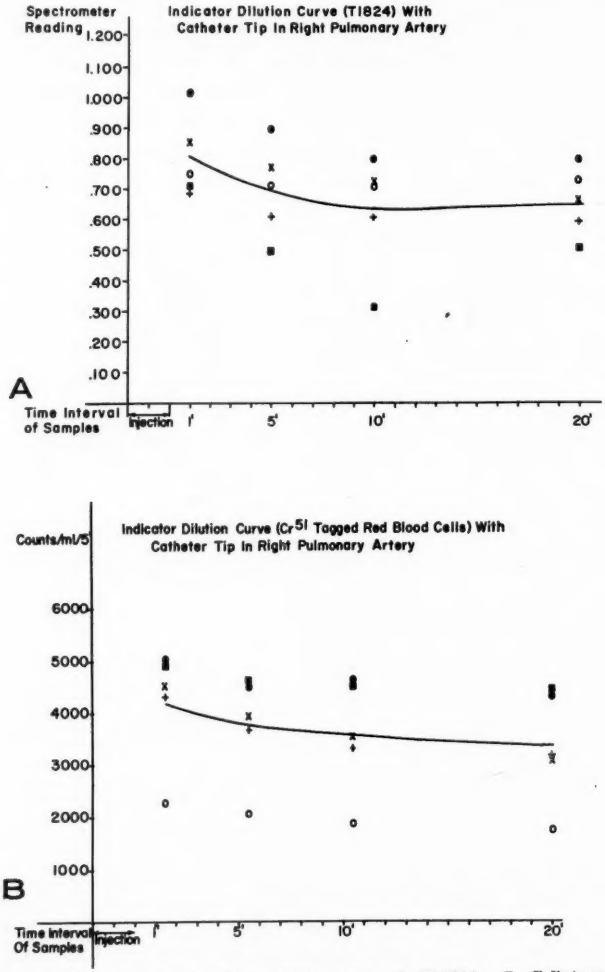


Fig. 2.—Indicator dilution curves in the control group. *A*, T-1824. *B*, Cr^{51} tagged red blood cells.

RESULTS

1. *Circulation of Indicator.*—In the control group, both indicators appeared immediately in the peripheral circulation in concentrations that declined from an initial high level to a lower plateau, 10 to 20 minutes after injection (Fig. 2).

In each group of the test series, the indicator dilution curves showed a delay in passage of the indicators through the lung, with a progressive leak into the systemic circulation. On releasing the pulmonary arterial occlusion, the indicator trapped in the lung was washed out into the systemic circulation, producing a sharp rise in concentration of the indicator, which then fell slightly to control levels as mixing occurred during the next 10 minutes.

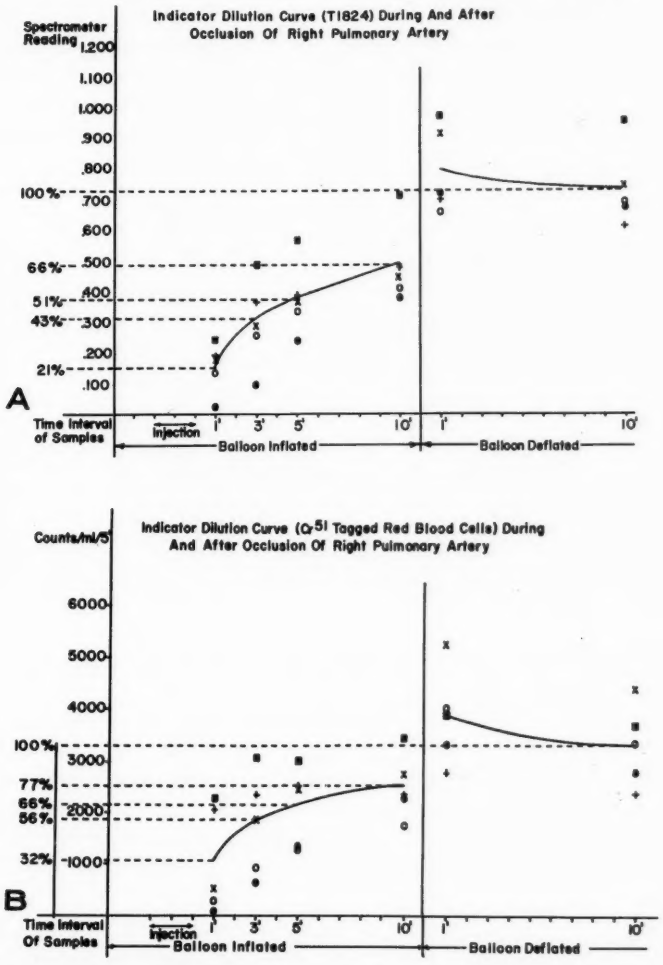


Fig. 3.—Indicator dilution curves in Group 2. *A*, T-1824. *B*, Cr^{51} tagged red blood cells. The linear plot allows the pulmonary-systemic leak to be expressed as a percentage of the total amount in solution, represented by the value 10 minutes after release of pulmonary arterial occlusion.

In Group 2, taking the average of values obtained with both indicators, the amount of the indicator reaching the systemic circulation at 1, 3, 5, and 10 minutes after injection was 26 per cent, 49 per cent, 58 per cent, and 71 per cent,

respectively (Fig. 3). In the remaining groups, the pulmonary-systemic leak was slightly smaller: the corresponding values being 26, 38, 44, and 65 per cent in Group 3, and 23, 34, 50, and 64 per cent in Group 4.

2. *Diffusion of Indicator.*—In the control group, the methylene blue stain appeared in the visceral pleura of all lobes of the lung within seconds of starting the injection. A uniform blue stain of maximum density was present at the end of the injection period, or within 2 minutes of completion of the injection. It began to fade at once and, 15 minutes after the injection, only a trace of the blue stain remained.

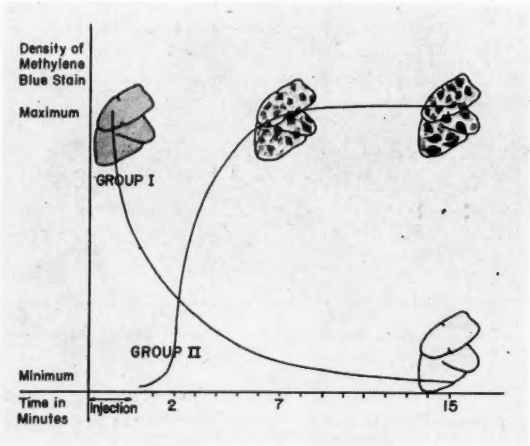


Fig. 4.—Schematic drawing which contrasts the even staining and rapid fading of the dye in the control group, with the slower, less uniform, but more persistant staining of the dye in the test series.

In the test series, the picture was quite different. In each group, the blue stain first appeared within 2 minutes of the end of the injection in all lobes, in patchy areas that stained more deeply than in the control group. A more uniform stain of lesser density then appeared, to reach a maximum 7 or 8 minutes after injection. There was no fading of the stain during the remainder of the 15-minute period of pulmonary arterial occlusion. There was no apparent difference in the distribution pattern of the dye between Groups 2, 3, and 4. The blue stain was never as uniform, either in density, or distribution, as in Group 1, the control series (Fig. 4).

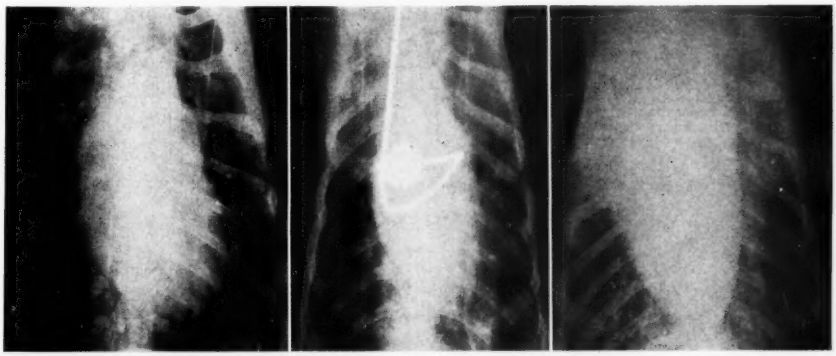
3. *Effect of Nitrogen Mustard.*—In the control series, all the animals survived the 4-week observation period. All developed transient anorexia, diarrhea, weight loss, and moderate leukopenia.

In the test series, none of the animals developed leukopenia. All the animals survived a dose of nitrogen mustard of 0.1 mg. or 0.2 mg. per kilogram. Only 1 animal survived a dose of 0.3 mg. per kilogram, and 1 a dose of 0.4 mg. per kilogram. Both of these developed transient anorexia, diarrhea, and weight loss.

All the animals developed roentgenographic evidence of pneumonitis or atelectasis within 24 hours of lung perfusion (Fig. 5). Roentgenographically there was no correlation between its extent, severity, or duration and the dose of nitrogen mustard. The pneumonitis cleared in 1 to 4 weeks in all but 2 animals.

CLINICAL EXPERIENCE

Right heart catheterization was performed four times on 3 patients. A No. 8½ Fr. Dotter-Lukas balloon-tipped double lumen cardiac catheter was introduced into a peripheral vein and positioned in the right or left pulmonary artery under fluoroscopic control. In 1 patient, a second catheter was inserted to record pressures proximal to the balloon. A Cournand needle was placed in the brachial artery.



A. *B.* *C.*

Fig. 5.—Roentgenograms of dog's chest. *A*, Before perfusion. *B*, During perfusion with the balloon catheter in position in the right pulmonary artery. *C*, Twenty-four hours later; showing severe penumonitis and atelectasis of the right upper lobe.

The balloon was inflated with 50 per cent Hypaque until the pulmonary artery pressure fell to pulmonary capillary levels (Figs. 6 and 7). Indicator dilution curves were then obtained by the method described, using 2 ml. of T-1824.

The balloon was then reinflated and the calculated dose of nitrogen mustard administered. Occlusion of the pulmonary artery was maintained for 15 minutes.

CASE REPORTS

CASE 1.—A dose of 0.1 mg. per kilogram of nitrogen mustard (7.5 mg. in 7.5 ml. of saline) was administered by this technique to a 54-year-old man with an inoperable epidermoid carcinoma of the right lower lobe, metastatic to the thyroid gland. A moderately severe pneumonitis developed within 24 hours. The patient remained asymptomatic and afebrile. Roentgenograms of the chest were made frequently until the patient's death 2 months later. There was no evidence of reduction in the size of the tumor. The pneumonitis did not completely clear (Fig. 8).

CASE 2.—A dose of 0.05 mg. per kilogram of nitrogen mustard (3.4 mg. in 6.8 ml. of saline) was administered to a 22-year-old man with bilateral pulmonary metastases from an osteogenic sarcoma of the right femur. Amputation had been performed a year previously. The left lung was perfused first and the right a month later. A mild perihilar pneumonitis that cleared in 48 hours appeared after the first perfusion. No pneumonitis was noted after the second perfusion. Following the first perfusion, the lesions in the left

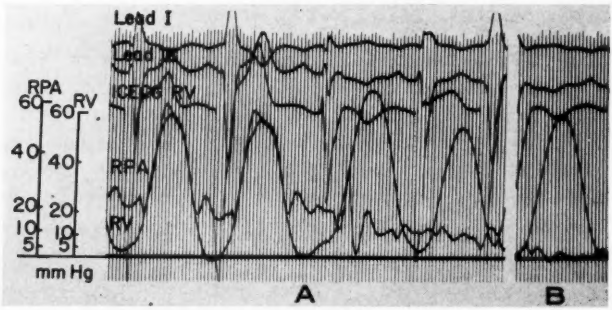
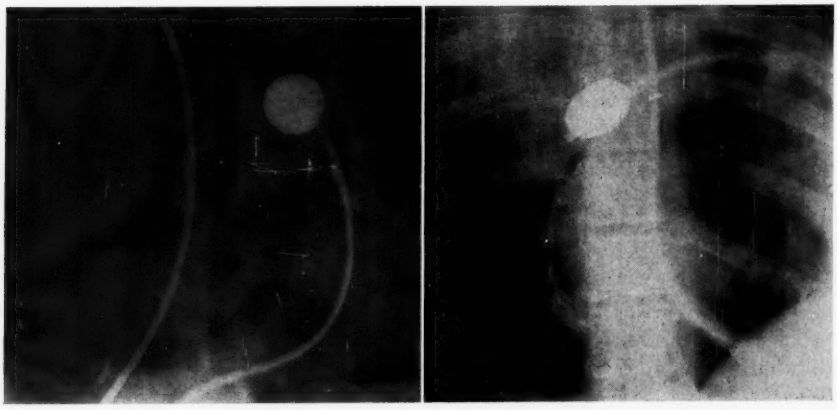


Fig. 6.—Case 3. Electrocardiographic and pressure data recorded at paper speed of 75 mm. per second. Time line interval, 0.02 second. *A*, At the moment of pulmonary arterial occlusion by the inflated balloon. *B*, Ten seconds later. The data show right bundle branch block and pulmonary hypertension. The pulmonary arterial pressure drops immediately to normal pulmonary capillary levels when occlusion of the artery is complete.



A. *B.*

Fig. 7.—*A*, Balloon catheter in left pulmonary artery. The balloon is seen end-on as the artery arches over the left main-stem bronchus. *B*, Balloon catheter in the right pulmonary artery. Here the balloon lies transversely and has a characteristic "sausage" shape when occlusion of the artery is complete.

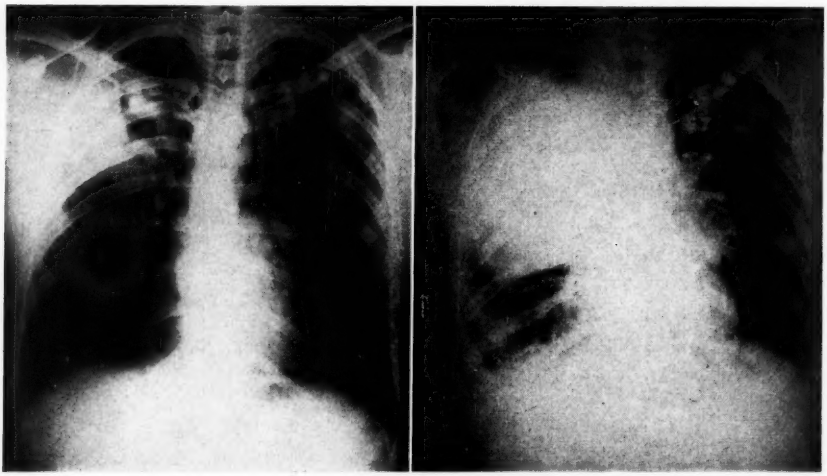
lung remained static with the exception of two that increased slightly in size in contrast to the rapid progression of those in the right lung (Fig. 9). Follow-up chest roentgenograms have not yet been obtained following perfusion of the right lung.

CASE 3.—A dose of 0.1 mg. per kilogram of nitrogen mustard (5.1 mg. in 5.1 ml. of saline) was administered to a 65-year-old man with an inoperable adenocarcinoma of the right lung, metastatic to the scalene nodes (Fig. 10, *A*). On admission to the George Washington University Hospital, the patient had developed partial atelectasis of the middle and lower lobes and a large pleural effusion, with roentgenographic evidence of lymphatic

A. *B.* *C.*

Fig. 8.—Case 1. Roentgenograms of the chest. *A*, Before perfusion. *B*, Twenty-four hours after perfusion. *C*, Two months after perfusion.

spread of the tumor throughout the right lung (Fig. 10, *B*). Thoracentesis on two occasions yielded small amounts of serosanguineous fluid. Examination for malignant cells was reported as grade 3 on the first specimen and negative on the second. Following the perfusion, the effusion rapidly absorbed and had not recurred at the time of the last follow-up roentgenogram made 6 weeks later (Fig. 10, *C*). Compared with the first, this last roentgenogram showed the parahilar mass to be slightly smaller; there was reaeration of the middle and lower lobes with persistent minimal pneumonitis, probably caused by the nitrogen mustard.



A. *B.*

Fig. 9.—Case 2. Roentgenograms of the chest. *A*, Before perfusion. *B*, One month after perfusion of the left lung.

DISCUSSION

These data show that an indicator substance, injected into the pulmonary artery distal to its occlusion, slowly diffuses throughout the lung and is gradually released into the systemic circulation, presumably as a result of eddy currents due to variations in the left atrial pressure. In the normal dog's lung, bronchial arterial flow and respiratory movements contribute very little to this circulatory pattern.

In our patients, as in those of Brofman[23] and Nemir[24] and their associates, the distal pulmonary arterial pressure fell to normal pulmonary capillary levels on inflation of the balloon (see Fig. 6). Oxygen studies on the last patient showed the saturation of this "lacunar" blood to be higher than that of the systemic blood. However, the indicator dilution curves in all 3 patients showed a substantially greater pulmonary-systemic leak than that found in the experimental animals (Fig. 11). Increased bronchial arterial flow in the presence of disease, without change in "lacunar" pressure, is a possible explanation, although this would not be expected in the presence of metastatic disease. Further study is needed to clarify this discrepancy.

The slow circulation and somewhat uneven distribution of the indicator explain the lower tolerance of the lung for nitrogen mustard administered in

A. *B.* *C.*

Fig. 10.—Case 3. Roentgenograms of the chest. *A*, One week before perfusion showing the right parahilar tumor, widening of the superior mediastinum, and minimal right pleural effusion. *B*, The day before perfusion, showing a large pleural effusion with partial atelectasis of the underlying middle and lower lobes. *C*, Six weeks after perfusion; the fluid has almost completely absorbed. There is aeration of the underlying lung, and the primary tumor is slightly smaller.

this manner than is found with isolated perfusion, which permits the safe administration of a dose of 0.4 mg. per kilogram.[2]

Study of the perfused dog's lung at autopsy showed that the acute pulmonary lesion produced by the nitrogen mustard, and interpreted roentgenographically as pneumonitis, was actually hemorrhagic necrosis of the lung. The chronic lesion in the human lung (Case 1) was found at autopsy to be anemic infarction secondary to widespread thrombosis of small pulmonary arteries. Neither lesion showed any inflammatory component.

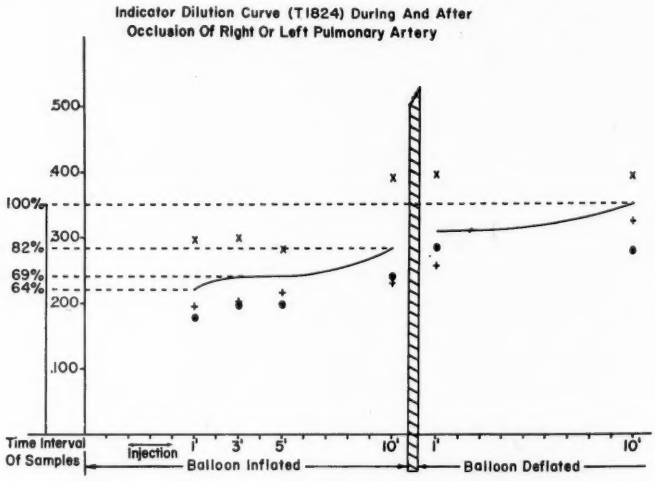


Fig. 11.—Cases 1, 2, and 3. Indicator dilution curve with T-1824.

The contrast in survival rate between the control and the test series of animals receiving nitrogen mustard, in a dose of 0.4 mg. per kilogram, suggests that the effect of the drug on the lung is the limiting factor in dosage rather than the size of the pulmonary-systemic leak. Experimental data suggest the maximum dose of nitrogen mustard to be 0.2 mg. per kilogram. Clinical experience, however, suggests that the maximum permissible dose may be 0.1 mg. per kilogram.

On a comparative weight basis (a lung to total body ratio of 1:150), allowing for the leak into the systemic circulation, a dose of nitrogen mustard administered in this way should provide a tissue concentration some fifty to one hundred times that obtainable by intravenous injection.

The results of treatment in 2 of the 3 patients are encouraging. Further study of this method, both with nitrogen mustard and other chemotherapeutic agents, such as 5-fluorouracil, is being undertaken.

SUMMARY

The circulation in the lung during unilateral pulmonary arterial occlusion by a balloon-tipped cardiac catheter has been studied in the dog and in man.

Studies with various indicators show widespread, slightly uneven, diffusion throughout the lung with a progressive leak into the systemic circulation.

The tolerance of the lung for nitrogen mustard administered by this technique is the limiting factor in dosage rather than the size of the pulmonary-systemic leak.

Nitrogen mustard, in doses of 0.05 to 0.1 mg. per kilogram, was safely injected into the pulmonary artery distal to its occlusion, with beneficial effect on primary and metastatic tumors in 2 of 3 patients.

This technique allows repeated chemotherapy of the lung without thoracotomy, is selective within limits, and, on a comparative weight basis (lung to total body), should provide a tissue concentration of nitrogen mustard fifty to one hundred times that obtainable by intravenous injection.

The assistance, in the indicator dilution studies, of Dr. George Kelser of the Department of Medicine and Dr. Ramsey MacCordy of the Department of Anesthesiology is gratefully acknowledged.

REFERENCES

1. Blades, B. B., and Hall, E. R.: Preliminary Observations on the Injection of Potentially Carcinolytic Drugs Into the Pulmonary Artery With Temporary Occlusion of the Blood Supply to the Lung, Am. Surgeon **24**: 492, 1958.
2. Pierpont, H., and Blades, B.: Lung Perfusion With Chemotherapeutic Agents, J. THORACIC SURG. **39**: 159, 1960.
3. Crockett, F. B., and Vass, C. C. N.: A Comparison of the Role of the Bronchial Arteries in Bronchiectasis and in Experimental Ligation of the Pulmonary Artery, Thorax **6**: 268, 1951.
4. Liebow, A. A., Hales, M. R., Bloomer, W. E., Harrison, W., and Lindskog, G. E.: Studies on the Lung After Ligation of the Pulmonary Artery, Am. J. Path. **26**: 177, 1950.
5. Liebow, A. A., Hales, M. R., Bloomer, W., and Lindskog, G. E.: The Genesis and Functional Implications of Collateral Circulation of the Lungs, Yale J. Biol. & Med. **22**: 637, 1950.
6. Wood, D. C., and Miller, M.: The Role of the Dual Pulmonary Circulation in Various Pathologic Conditions of the Lungs, J. THORACIC SURG. **7**: 649, 1938.
7. Cudcowicz, L., and Wraith, D. G.: A Method of Study of the Pulmonary Circulation in Finger Clubbing, Thorax **12**: 313, 1957.
8. Roosenburg, J. G., and Deenstra, H.: Bronchial-Pulmonary Vascular Shunts in Chronic Pulmonary Infections, Dis. Chest **26**: 664, 1954.
9. Gilroy, J. C., Wilson, V. H., and Marchand, P.: Observations on the Hemodynamics of Pulmonary and Lobar Atelectasis, Thorax **6**: 137, 1951.
10. Wright, D.: The Blood Supply of Abnormal Tissues in the Lungs, J. Path. & Bact. **47**: 489, 1938.
11. Marchand, P., Gilroy, J. D., and Wilson, V. H.: An Anatomical Study of the Bronchial Vascular System and Its Variations in Disease, Thorax **5**: 207, 1950.
12. Cudcowicz, L., and Armstrong, J. B.: The Bronchial Arteries in Pulmonary Emphysema, Thorax **8**: 46, 1953.
13. Miller, W. S.: The Lung, Springfield, Ill., 1950, Charles C Thomas, publisher.
14. Berry, J. L., and de Burgh Daly, I.: The Relation Between the Pulmonary and Bronchial Vascular Systems, Proc. Roy. Soc., s B, London **109**: 319, 1931.
15. Ghoreyeb, A. A., and Karsner, M. T.: A Study of the Relation of Pulmonary and Bronchial Circulation, J. Exper. Med. **18**: 500, 1913.
16. Verloop, M. C.: The Arteriae Bronchiales and Their Anastomoses With the Arteria Pulmonalis in the Human Lung: A Microanatomical Study, Acta anat. **5**: 171, 1948.
17. Gray, F. D., Lurie, P. R., and Whittemore, R.: Circulatory Changes in Chronic Pulmonary Disease, Yale J. Biol. & Med. **23**: 380, 1951.
18. Steinberg, I., and Robb, G. P.: Mediastinal and Hilar Angiography in Pulmonary Disease, Am. Rev. Tubere. **38**: 557, 1938.
19. Spatt, S. D., and Grayzel, D. M.: Cor Pulmonale, Am. J. Med. **5**: 252, 1948.

20. Kountz, W. B., Alexander, M. L., and Prinzmetal, M.: The Heart in Emphysema, Am. Heart J. **11**: 163, 1936.
21. Scott, R. W., and Garvin, C. F.: Cor Pulmonale: Observations in Fifty Autopsy Cases, Am. Heart J. **22**: 56, 1941.
22. Spain, D. M., and Handler, B. J.: Chronic Cor Pulmonale, Arch. Int. Med. **77**: 37, 1946.
23. Brofman, B. L., Charms, B. L., Kohn, P. M., Elder, J., and Rizika, M.: Unilateral Pulmonary Artery Occlusion in Man, J. THORACIC SURG. **34**: 206, 1957.
24. Nemir, P., Stone, H. H., Hawthorne, H. R., and Mackrell, T. N.: Further Experiences With the Method of Controlled Unilateral Pulmonary Artery Occlusion in the Study of Lung Function, J. THORACIC SURG. **32**: 563, 1956.
25. Creech, O., Krementz, E. T., Ryan, R. F., and Winblad, J. N.: Chemotherapy of Cancer, Ann. Surg. **148**: 616, 1958.
26. Hamilton, W. F., Moore, J. W., Kinsman, J. M., and Spurling, R. G.: Studies on the Circulation. IV., Am. J. Physiol. **99**: 534, 1932.
27. Spencer, W. A., Thistlethwaite, T. R., and Albert, S. N.: A Simplified Method for Blood Volume Determination Using Radioactive Isotopes, S. Forum **7**: 3, 1956.

(For Discussion, see page 699)

AN EXPERIMENTAL STUDY OF PULMONARY ARTERY REPLACEMENT

Joseph W. Gilbert, M.D. (by invitation), William P. Cornell, M.D. (by invitation), and Theodore Cooper, M.D., Ph.D. (by invitation), Bethesda, Md.

THE CORRECTION of certain congenital cardiovascular malformations, such as atresia of the main pulmonary artery, varieties of persistent truncus arteriosus, and transposition of the great vessels, is contingent upon effecting communication between the right ventricle and the pulmonary arterial tree. Replacement of the bifurcation of the pulmonary artery distal to the valve with an aortic homograft was undertaken by Robinson and his associates[1] in exploring means for treating thrombosis of the pulmonary arteries. They concluded that this technique was unsatisfactory since degeneration of the grafts was evident as early as 70 days postoperatively. Donovan and his co-workers[2] employed venous-lined tubes of polyethylene or homologous trachea between the right ventricle and the left pulmonary artery; the lumens usually became obstructed within 3 or 4 weeks. The technical problems and the hemodynamic consequences attending insertion of valveless Edwards-Tapp Teflon prostheses between the right ventricle and a main branch of the pulmonary artery comprise the subject of this study.

METHOD

Adult mongrel dogs, weighing 13 to 17 kilograms, were anesthetized with thiopental sodium (30 mg. per kilogram) and the lungs were mechanically ventilated through a cuffed intratracheal tube with 100 per cent oxygen. Digoxin, 0.015 mg. per kilogram to 0.03 mg. per kilogram, was administered intravenously following induction of anesthesia and the left or right thorax was entered through the fourth intercostal space (Fig. 1). Pressures in the femoral artery, right ventricle, and a subsegmental tributary of the ipsilateral pulmonary artery were measured by means of Statham transducers and inscribed, with Lead II of the electrocardiogram, on a multichannel recorder. The right or left pulmonary artery was mobilized and divided and an Edwards-Tapp prosthesis of woven Teflon, 5/16 to 3/8 inch in diameter and 6 to 8 cm. in length, was approximated to its distal end by means of a continuous 5-0 arterial suture. The proximal end, supported by an inclosed extraluminal polyethylene sleeve, was inserted into the outflow tract of the right ventricle through a purse-string suture during 30 to 60 seconds of caval occlusion. The prosthesis was fixed to the myocardium by means of additional interrupted sutures. The thorax was closed anatomically.

From the Clinic of Surgery, National Heart Institute, Bethesda 14, Md.

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

Right heart catheterization and angiocardiography were carried out in all survivors 3 to 90 days after operation. Serial radiographs (2 per second) were made following the selective right ventricular injection of 70 per cent Urokon (1 c.c./Kg.). In all animals in which initial patency of the prosthesis was demonstrated, the study was repeated after an interval of at least 90 days (Fig. 2). Dogs in which the grafts were shown to be obstructed were sacrificed and the causes of failure determined. Three animals having patent grafts were sacrificed at intervals of 5 days to 171 days to observe the lumens, anastomoses, and ventricular orifices of the prostheses.

RESULTS

Fifteen of the 25 dogs in which the procedure was performed survived. Two died during operations because of technical errors. Delayed hemorrhage from ventricular detachment of the prosthesis (2 dogs) and disruption of the pulmonary artery anastomosis (1 dog) accounted for 3 deaths. Massive postoperative atelectasis resulted in the loss of 3 dogs and infection caused the death of 2. In 1 animal, which died with multiple pulmonary abscesses, the prosthesis was obstructed at the pulmonary artery suture line by thrombus. The grafts were patent in the 4 remaining dogs in which death could not be ascribed to technical inadequacy.

The acute circulatory changes associated with insertion of the valveless unilateral prosthesis are illustrated in Fig. 3. Moderate elevation of systolic pressure and slight elevation of end-diastolic pressure occurred in the right ventricle. The pulse pressure was increased in the pulmonary artery distal to the graft, the cardiac rate was increased, and the systemic arterial pressure remained at control level.

Patency of the prosthesis was demonstrated in 10 of the 15 survivors at the time of the first study, 3 to 90 days postoperatively. The causes of early obstruction were kinking because of excessive graft length or atelectasis in three instances, improper ventricular insertion in one, and infection in one. Four prostheses which were initially patent were found to be obstructed at 3 months. In three, improper right ventricular insertion was responsible for obstruction, and in one, infection.

The lumens of patent grafts were lined by thin fibrinous films at 5 days, and by 170 days this had become a densely adherent gray membrane. Neither the suture lines nor the ventricular orifices showed evidence of stenosis (Fig. 4).

TABLE I. POSTOPERATIVE RIGHT HEART CATHETERIZATION DATA

DOG NO.	POSTOP. INTERVAL (DAYS)	F.A. (MM. Hg)	R.A. (MM. Hg)	R.V. (MM. Hg)	P.A. S/D, MEAN (MM. Hg)	HEART RATE
472	3	160/100	3-9	32/5		148
502	6	140/100	4-8	36/6		140
582	19	172/122	3-7	40/6		164
	90	164/110	4-9	38/5		160
975	49	135/94	3-6	36/5		142
	90	193/147	4-9	35/5	28/6, 14	180
832	90	186/102	4-7	38/8		140
Anesthetized normal		150/100		26/6		130

Legend: F.A. = femoral artery. R.A. = right atrium. R.V. = right ventricle. P.A. = pulmonary artery (contralateral).

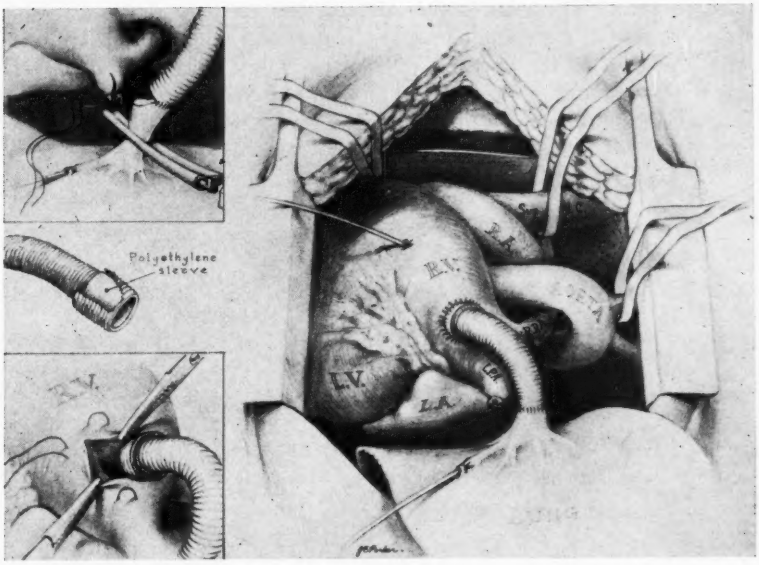


Fig. 1.—Method employed in replacement of the left main pulmonary artery with woven Teflon graft. The proximal end of the prosthesis is inserted into the right ventricle during brief caval occlusion.

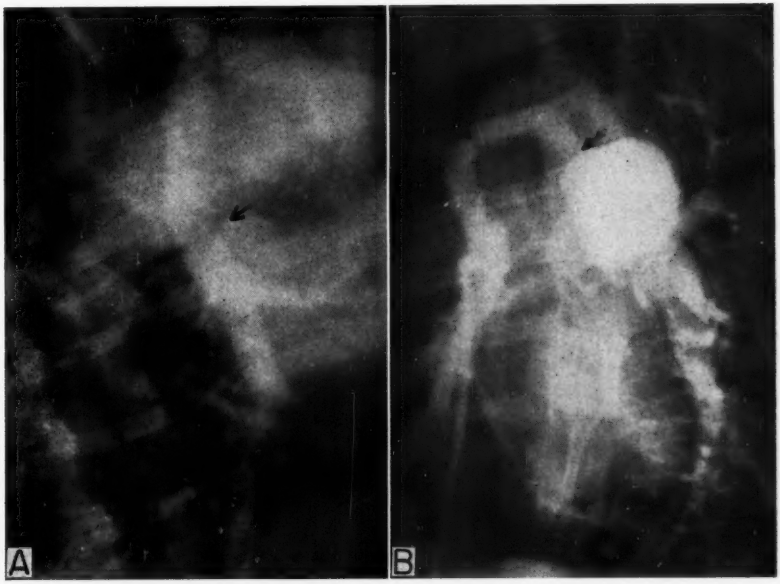


Fig. 2.—Angiocardiogram showing patent prosthesis between right ventricle and right pulmonary artery *(A)* in lateral view at 19 days and *(B)* in anteroposterior view at 90 days.

Postoperative right heart catheterization data comprise Table I. Minimal elevation was seen in the systolic pressure in the right ventricle; the systolic and diastolic pressures in the opposite pulmonary artery were normal. Systemic arterial hypertension and tachycardia of moderate degree were present in all animals, which were under light thiopental sodium anesthesia.

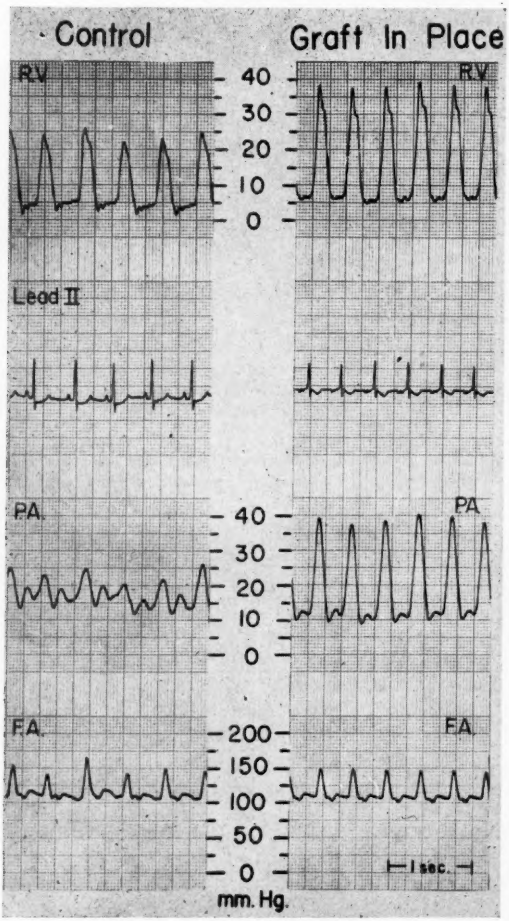


Fig. 3.—Acute circulatory changes associated with the insertion of a valveless graft between the right ventricle and the left pulmonary artery. *R.V.* = right ventricle; *P.A.* = pulmonary artery; *F.A.* = femoral artery.

COMMENT

The replacement of portions of the main pulmonary arterial tree by means of Edwards-Tapp Teflon prosthesis seems feasible. Although the use of such material for this purpose appears to afford certain advantages over venous or aortic homografts, proper evaluation of its ultimate fate in the lesser circulation requires more prolonged observation. Unlike grafts inserted in systemic vessels,

pulmonary artery replacements must traverse the mediastinum virtually unsupported by adjoining structures. This, together with the low pressure in the pulmonary artery, predisposes toward obstruction by kinking, especially in the event of atelectasis. Despite the relative sparsity of adjacent tissues, intraluminal healing of the Teflon prostheses appears to progress in accordance with the process described[3] following their insertion in systemic arteries. Stricture at the site of anastomosis, which has been reported after synthetic graft replacement of segments of the inferior vena cava,[4-7] has not been observed in this experience. No histologic changes have been discernible in the vessels or parenchyma of the lung distal to the valveless grafts.

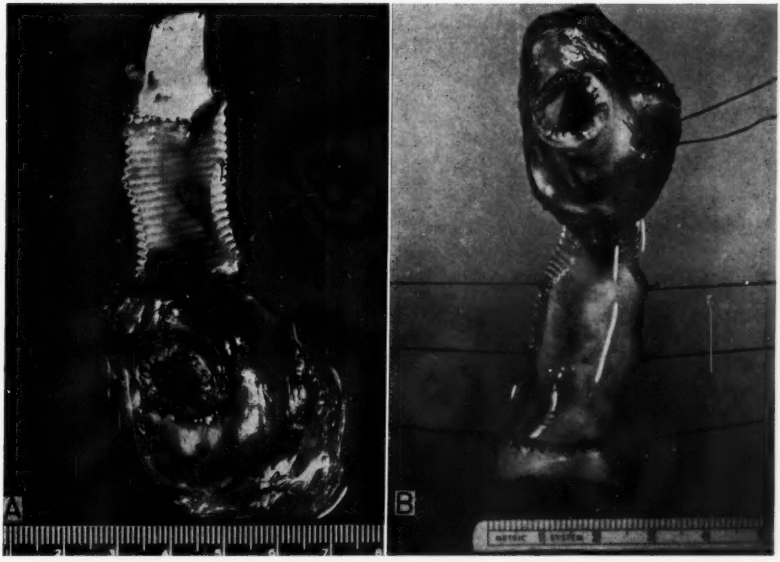


Fig. 4.—Teflon prostheses from dogs sacrificed *(A)* at 5 days and *(B)* at 171 days following insertion.

Pressure measurements in the right ventricle demonstrate small but significant changes in systolic and end-diastolic pressures following insertion of a valveless unilateral pulmonary artery prosthesis and dilatation of this cardiac chamber was regularly noted. These findings reflect the increased end-diastolic volume and stroke output caused by regurgitation from one lung. The use of a graft of excessive length increases the resistance to flow to the respective lung, resulting in further elevation of right ventricular pressure. Increasing the flow through the prosthesis by occluding the opposite pulmonary artery accentuates this gradient and the elevations in systolic and end-diastolic pressures in the right ventricle. These observations emphasize the critical factors of graft length and proportion which must be considered in replacement of the main pulmonary artery and its bifurcation. Although the infliction of regurgitation in the performance of pulmonary valvulotomy has not generally been accorded serious

concern, Fowler and Duchesne[8] have shown experimentally that the lesion may not be entirely innocuous. Replacement of the pulmonary artery with a graft which is inelastic, as well as valveless, imposes a more marked burden upon the right ventricle. The availability of a satisfactory prosthetic valve will significantly simplify this problem. Finally, it must also be recognized that the individual variables which may affect the hemodynamic consequences of pulmonary artery replacement in a patient with congenital heart disease may not necessarily be anticipated from studies performed in the normal dog.

SUMMARY

Woven Edwards-Tapp Teflon blood vessel prostheses were inserted between the right ventricle and a main branch of the pulmonary artery in 25 dogs, 15 of which survived chronically. Right heart catheterization and selective angiocardiography were performed in the survivors and in 10 the grafts were found to be patent 5 days to 90 days postoperatively. Repeat studies disclosed that in 4 animals, in which the grafts were initially patent, obstruction subsequently occurred within 90 days. The causes of failure, certain pathologic findings, and hemodynamic observations pertinent to this use of vascular prostheses are considered.

REFERENCES

1. Robinson, G., Glotzer, P., Gilbert, M., and Hurwitt, E. S.: Aortic Homograft Replacement of the Main Pulmonary Artery, J. THORACIC SURG. **36**: 555, 1958.
2. Donovan, T. J., Hufnagel, C. A., and Eastcott, H. H. G.: Techniques of Endocardial Anastomosis for Circumventing the Pulmonic Valve, J. THORACIC SURG. **23**: 348, 1952.
3. Edwards, W. S., Boyles, P., Forrest, C., and Waibel, P.: Progress in Synthetic Graft Development. An Improved Crimped Graft of Teflon, Surgery **45**: 298, 1959.
4. Deterling, R. A., and Bhonslay, S.: Use of Vessel Grafts and Prostheses for Relief of Superior Vena Caval Obstruction, Surgery **38**: 1008, 1955.
5. Ohara, I., and Sakai, T.: Transplantation of the Large Venous System With Various Blood Vessel Substitutes, Surgery **42**: 928, 1957.
6. MacLean, L. D., Phibbs, C. M., Flom, R. S., and Brainard, J. B.: Replacement of Vital Veins: An Experimental Study, Ann. Surg. **149**: 549, 1959.
7. Sauvage, L. R., and Wesolowski, S. A.: Anastomoses and Grafts in the Venous System With Special Reference to Growth Changes, Surgery **37**: 714, 1955.
8. Fowler, N. O., and Duchesne, E. R.: Effect of Experimental Pulmonary Valvular Insufficiency on the Circulation, J. THORACIC SURG. **35**: 643, 1958.

(For Discussion, see page 699)

A SIMPLE STAPLING DEVICE FOR THE ANASTOMOSIS OF BLOOD VESSELS

Timothy Takaro, M.D., Oteen, N. C.

A NUMBER of instruments have recently been described for the anastomosis of blood vessels by means of the simultaneous application of multiple small staples circumferentially.[1-5] While the usefulness of such devices, either experimentally or clinically, has not yet been established, there is certainly room for the opinion that instruments which can perform a technically perfect anastomosis speedily at every application may ultimately find utility, especially in small blood vessel anastomoses and in organ transplantation. Therefore, basic experimental studies of the use of such instruments seem to be indicated.

A device of but few components and of simple design has been constructed which has made possible the mechanical anastomosis of prosthetic grafts to blood vessels by intubation of the blood vessel, the stapling being carried out while blood is flowing through the junction between the vessel and the graft. This instrument, the technique used, and the results of laboratory experiments with this technique are described below.

DESCRIPTION OF DEVICE

One jaw of a previously described cylindrical vessel coupling device,[6, 7] bivalved to permit removal, was modified by fitting it with eight tiny shafts for housing staples and for guiding two four-pronged staple drivers (Figs. 1 and 2). On the opposing jaw, eight, precisely placed, specially shaped indentations for clinching the staples were fashioned. A circular bivalved nut which was fitted onto the staple-housing unit served to force the staple driver prongs into the shafts, thereby pressing eight tiny staples across the interval between the clincher unit and the staple-housing unit, and causing the legs of the staples to be bent inward toward each other. The staples were made by hand of surgical stainless steel wire, or dental gold clasp wire, of 0.012 in. diameter, cut to produce a sharp point on each leg (Fig. 3). Leg lengths of the staples varied from 0.074 to 0.125 in. The width of the staples at the base was 0.086 in. The internal diameter of the staple-housing unit was 9 mm. and the external diameter, 12 mm.

METHOD

Elasticized Dacron grafts* were interposed in the thoracic aorta of 25 normothermic dogs, weighing 13 to 25 kilograms, using the stapling device just described for effecting one

From the Cardiopulmonary Research Laboratory, Veterans Administration Hospital, Oteen, N. C.

This project was aided by a grant from the American Heart Association.

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

*"Fluflon" Dacron, manufactured by the Bentley, Harris Manufacturing Company, Conshohocken, Pa. Two different sizes were used, measuring 13 mm. and 17 mm. flat.

anastomosis (Fig. 4). The other was sutured with arterial silk, with the aid of a previously described coupling device.[7] The staple-housing unit was first loaded with eight staples. Next, the two four-pronged staple drivers were engaged in the staple housing shafts. Then one end of an elasticized Dacron prosthesis was passed through the lumen of the staple-housing unit and cuffed back upon it, covering the openings of the staple-housing shafts. The other end of the graft was cuffed back upon the graft-bearing portion of the other coupling device. The prosthesis was preclotted with fresh blood.

The animals were anesthetized with Nembutal and the trachea was intubated. A positive pressure respirator was attached. The left pleural cavity was entered through the fifth

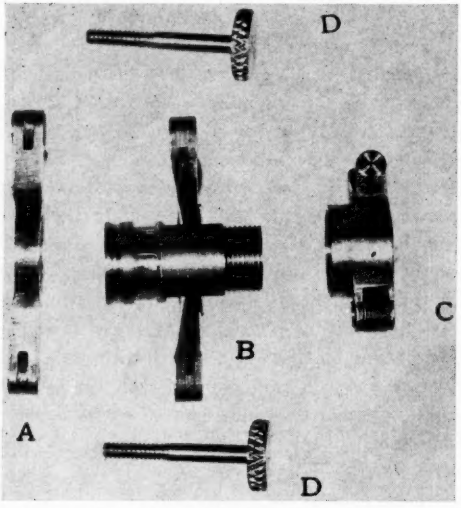


Fig. 1.—Stapling device disassembled, side view.

A, Clincher unit, hinged to allow removal.

B, Staple-housing unit, with staple drivers in place. This unit is also hinged. Note shallow groove near clincher end.

C, Staple-driving nut, hinged to permit removal.

D, Bolts to approximate clincher and staple-housing units.

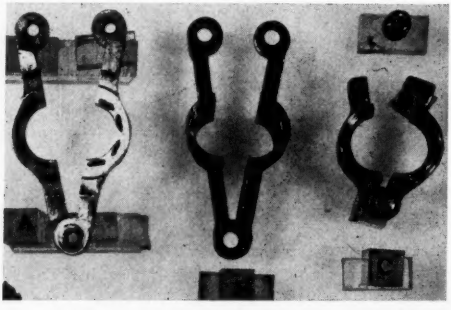


Fig. 2.—Stapling device, disassembled, end-on view.

A, Clincher unit with jaws opened showing four depressions engraved in each semicircular jaw to clinch staples.

B, Staple-housing unit with jaws opened showing four staple-housing shafts positioned exactly opposite the engraved depressions on the clincher unit jaws. Staple drivers and staples have been removed.

C, Circular staple-driving nut opened with fastening bolt removed and shown above the nut.

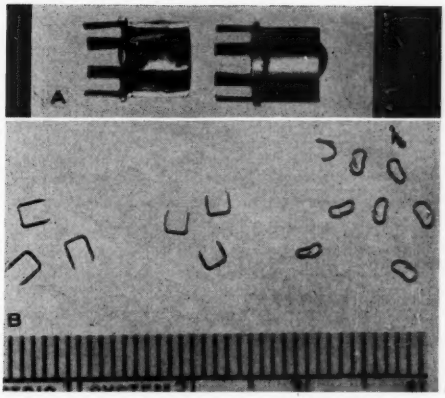


Fig. 3.—*A*, Close-up of the 2 semi-circular staple drivers, the four prongs of which fit exactly into the four staple-housing shafts of each jaw of staple-housing unit.

B, Close-up of staples. At *left*, long-legged staples; in *middle*, short-legged staples (see text); at *right*, staples which have been recovered from an aortic graft anastomosis, by digesting away the tissues, to show shape of clinched staples. One at top was improperly deformed.

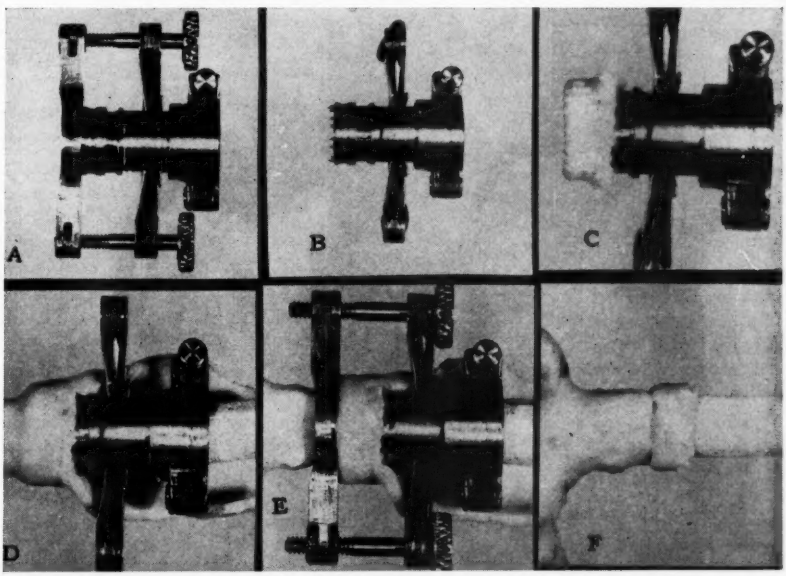


Fig. 4.—Steps in technique of stapling prosthesis to aorta.

A, Assembled stapler, side view.

B, Stapler partially disassembled. Staple-housing unit loaded with staples, the points of which are just visible, protruding from staple shafts for demonstration only. In use, they are retracted within the shafts, out of sight. Note staple drivers in place.

C, Dacron graft pulled through loaded staple-housing unit and cuffed back upon the groove overlying staple shafts.

D, Everted graft inserted into aortic lumen. It is held in place temporarily by a wire loop held in a tonsil snare (not shown), until bolts can be applied. Ordinarily, clincher unit (also not shown) has already been positioned around the intact aorta.

E, Bolts passed through staple-housing unit arms have engaged clincher unit arms and approximated the jaws of the stapler, locking aorta and graft in the interval between the jaws. The circular staple-driving nut has been applied and tightened against the staple drivers, forcing the eight staples across the interval between the jaws of the stapler and clinching them, completing the mechanical anastomosis.

F, The stapler has been removed by disengaging the two main bolts, as well as the bolt fastening the circular nut, and opening the 3 major components on their hinges. This shows the appearance of the anastomosis after trimming off excess aorta.

interspace; the mediastinal pleura was incised and reflected back from the aorta. The first five pairs of intercostal arteries were divided between ligatures, mobilizing the proximal descending thoracic aorta. The appropriate semi-circular opposing jaws of each coupling device were placed about the intact aorta near the proximal and distal ends of the isolated segment and the matching pairs fastened together to complete the circle. A holding suture of 5-0 arterial silk was placed near the proximal and distal ends of the isolated, intact aorta, and two loops of braided wire were placed around the aorta and tied onto tonsil snares, which were held ready for tightening. The aorta was then cross-clamped just distal to the subclavian artery and also at the level of the sixth intercostal artery, and the segment of aorta between the clamps was incised longitudinally.

The staple-housing unit bearing the everted Dacron prosthesis was inserted into the lumen of the aorta at one end of the isolated segment, through the incision in the aortic wall. In order to keep the device from slipping out again while the approximating bolts were being applied, the device was held firmly in place in the aortic lumen by tightening the wire loop around that portion of the aorta which hugged the everted cuff of prosthesis. The two bolts were then slipped through the holes in the arms of the staple-housing unit, engaged in the holes in the arms of the opposing staple-clinching unit, and tightened a determinate distance, sufficient to close the gap between the two circular jaws of the stapling device and to lock the aorta and the graft together in the interval between the jaws, but not sufficient to allow crushing of the aortic wall.

A similar procedure was carried out at the other end of the isolated segment of aorta. Aortic continuity was thus restored by an indwelling Dacron prosthesis which was locked into place proximally and distally by coupling devices. The aortic occluding clamps were removed, restoring blood flow through the thoracic aorta. The wire loops were loosened and removed. The segment of incised aorta between the two coupling devices was resected.

The circular staple-driving nut was applied to the staple-housing unit and fastened in place with a small bolt. This circular nut was then turned clockwise on the unit, pushing the two staple drivers ahead, and forcing the eight small staples simultaneously through the graft and aortic walls and on into the indentations in the clincher unit. Further tightening of the nut with the aid of strong forceps caused the staples to be "clinched," that is, the staple legs were bent inward toward each other. This completed the mechanical anastomosis. The bolts approximating the 2 circular jaws were removed and the bivalved circular jaws of the stapler were opened and also removed. Bleeding between the staples was controlled by wrapping the anastomosis in gauze and applying moderate pressure. Occasionally a few sutures of silk or a second row of staples had to be applied to control bleeding. The distal anastomosis was sutured with 5-0 arterial silk in the usual manner, after which this vessel couple was also removed, leaving the stapled and sutured prosthesis in the mediastinum. Following closure of the mediastinum over the graft, the chest was closed and drained for a few hours by a negative pressure suction pump. Occasionally a blood transfusion was given during surgery. Antibiotics were sometimes used.

In 6 animals, for the reason just noted, two rows of staples, staggered with respect to each other, were placed. In 20 animals, the stapled anastomosis was the proximal one, while in 5, it was the distal anastomosis. Upon sacrifice or death of the animal, an autopsy was done and the engrafted aorta was recovered, opened, inspected, and photographed. Sections were taken across each anastomosis for histologic examination, after removal of the staples by fine wire cutters. The sutured anastomosis served as the control. From some animals, sections of both the stapled and sutured anastomoses, 1 cm. in width, were prepared and subjected to tensile strength measurements, using the inclined plane Scott tester,* the breaking point of the anastomosis being used as the end point. In 8 animals, thoracic aortography was performed at varying intervals following surgery.

*Made available through the courtesy of the American Enka Corporation, Enka, N. C.

RESULTS

The procedure was carried out in 25 animals. The average time of aortic occlusion needed to interpose the graft and to lock it into place, re-establishing blood flow, was 5½ minutes. Aortas, ranging in diameter from 1.3 to 2.0 cm. in the blood-filled state, were successfully stapled with the single model available. There were two immediate failures, one of which resulted in death of the animal on the table. Five animals died at intervals of 1 day to 4 months after surgery of hemorrhage, infection, or pneumonia. In 2 of these, the graft was occluded. The remaining animals, including 3 which developed paralysis of the hindquarters, were sacrificed at intervals from 1 week to 8 months or

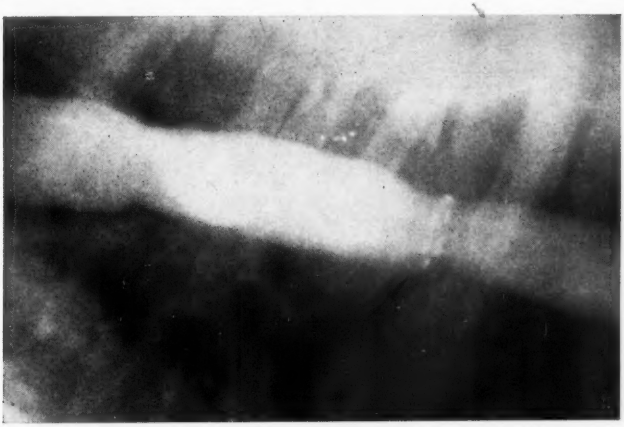


Fig. 5.—Thoracic aortogram, Animal 17, showing Dacron graft interposed in upper descending thoracic aorta. The proximal anastomosis, indicated by slight narrowing, was made with silk sutures with the aid of an Oteen vessel coupling device. The distal anastomosis was accomplished by the use of the stapling device and is indicated by the arrow.

were allowed to survive, 8 being kept for long-term study (see Table I). Of the 25 instances, the graft was patent in all but 2 as shown at autopsy, upon aortography or by palpation of femoral pulses. Aortograms showed varying degrees of narrowing at both anastomoses, ranging from slight to marked, depending upon the disparity between the diameter of the blood-filled aorta and the diameter of the graft (Fig. 5). Calculation of the area of the aortic lumen, proximal to the stapled anastomosis and at the anastomosis, showed a reduction in area up to 75 per cent in a few instances, although the area at the anastomosis averaged only 30 per cent less than the area of the lumen of the graft itself.

The results of thirty-one tests of the breaking point of the anastomoses performed on the inclined plane tester favored the silk sutured anastomoses. This was true of the specimens from 3 animals sacrificed within 2 weeks of operation, as well as from 5 animals allowed to survive up to 8 months. The average breaking point of stapled anastomoses occurred at 2.6 pounds of stretch (range 1.2 to 5.2) compared with an average of 3.3 for silk (range 1.0 to 6.1).

TABLE I

NUMBER IN SERIES	NUMBER OF ROWS OF STAPLES AND SITE OF STAPLED ANASTOMOSIS	TIME OF AORTIC OCCLUSION (MINUTES)	BLEEDING FROM STAPLED ANASTOMOSIS	DURATION OF SURVIVAL (DAYS)	FATE OF ANIMAL	REMARKS
1	1 row Proximal	6	Minimal	120	Died	Graft thrombosed; micro abscesses around proximal anastomosis
2	1 row Proximal	8	Excessive	90	Sacrificed	Graft patent; small patch of fibrin deposit, distal portion of graft
3	1 row Proximal	5	Excessive	270	Alive	Animal in good health
4	1 row Proximal	7½	—	—	Technical failure	Clincher put on backward; operation changed; animal lost to this study
5	1 row Proximal	4½	Minimal	250	Sacrificed	Graft patent with smooth glistening lining of compact fibrous tissue
6	1 row Proximal	4½ + 24*	Excessive	8	Sacrificed	New batch of staples, too stiff to clinch; prolonged occlusion to reload stapler resulted in paralysis of hind limbs
7	1 row Proximal	4	Excessive	—	Died at operation	Technical failure: prosthesis too thick, staples too short to clinch; hemorrhage on table
8	1 row Proximal	5½	Minimal	105	Sacrificed	Graft patent; thick deposits of fibrin, distal ⅔ of graft
9	1 row Proximal	5½	Excessive	60	Died	Graft thrombosed; gross infection with abscess formation rupturing into esophagus
10	1 row Proximal	4	Excessive	21	Died	Graft patent; animal died of pneumonia
11	1 row Distal	5½	Moderate	30	Died	Graft patent; cause of death unknown; minimal inflammatory reaction
12	2 rows Proximal	6	Moderate	225	Alive	Animal in good health; moderate narrowing of anastomoses on aortogram
13	2 rows Proximal	5½	Moderate	221	Alive	Animal in good health; moderate narrowing of anastomoses on aortogram

14	2 rows Proximal	4	Minimal	180	Sacrificed	Graft patent; thick deposits of fibrin, middle half of graft
15	2 rows Proximal	8	Excessive	214	Alive	Animal in good health
16	2 rows Distal	5	Excessive	3	Sacrificed	Became paralyzed 3 days postop.; thrombus at pocketed distal anastomosis led to probable aortic embolus; graft patent
17	1 row Distal	7½	Excessive	120	Alive	Animal in good health
18	1 row Distal	4½	Excessive	80	Sacrificed	Graft patent; smooth thin lining; gross infection with abscess formation rupturing into esophagus
19	1 row Proximal	4½	Moderate	102	Alive	Animal in good health
20	2 rows Distal	3½	Moderate	98	Alive	Animal in good health
21	1 row Proximal	6	Excessive	45	Alive	Animal in good health
22	1 row Proximal	10 + 10*	Excessive	35	Sacrificed	Longitudinal tear in aorta required prolonged occlusion to suture; animal paralyzed in hindquarters postop. but began to recover; graft patent
23	1 row Proximal	3½	Moderate	14	Sacrificed	Graft patent
24	1 row Proximal	4	Excessive	1	Died	Died of massive hemorrhage between staples 1 day postop.; graft patent, anastomoses intact
25	1 row Proximal	5	Excessive	5	Sacrificed	Graft patent

*A second period of occlusion was necessary to reload stapler or to control hemorrhage.

PATHOLOGY

Grossly, there was a rather obvious ridge at the stapled anastomosis within the aortic lumen in most specimens, where the graft was cuffed back upon itself. This resulted in moderate narrowing of the lumen. Fibrin collected at the base of the ridge early; later, fibrous tissue bridged the gap between the aortic intima and the inner surface of the graft. This was usually not as well developed or as smooth as the union between the graft and the aorta at the sutured anastomosis. There was also a less obvious ridge and less narrowing of the

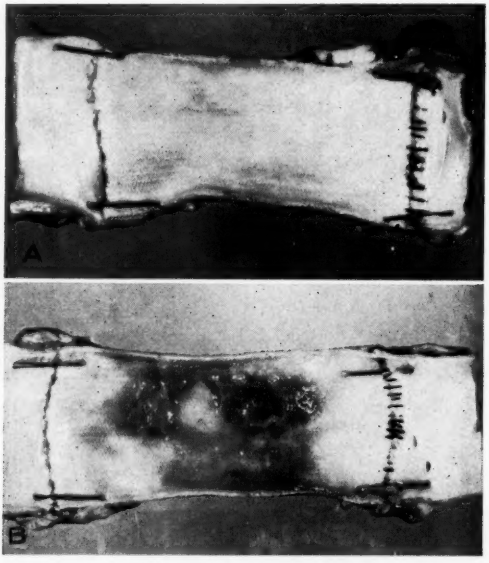


Fig. 6.—Gross specimens of stapled aortic Dacron grafts.

A, Graft from animal sacrificed 8 months after operation; the staples are scarcely visible and are covered with thin smooth fibrous tissue as is the sutured anastomosis at the other end of the graft.

B, Graft from animal sacrificed 6 months after operation which shows deposits of fibrin, several millimeters in thickness, in mid-portion of graft.

lumen at the sutured anastomosis. There was little or no inflammatory reaction in the tissues surrounding the holes which remained after removal of the staples. The elastic fibers were compressed at the site of the staples, but not in the areas between staples. Occasionally there was disruption of elastic fibers at the anastomotic site, probably from excessive compression of the media by the stapling device. In contrast to the stapled anastomosis, there was usually a mild to moderate inflammatory reaction around the silk threads at the sutured anastomosis. Compression and, less commonly, disruption of elastic fibers, presumably due to the coupling device, were seen at this anastomosis also.

The grafts were lined in the immediate postoperative period with fibrin, which later became organized into a compact layer of fibrous tissue, grossly smooth and glistening (Fig. 6). However, in several specimens, deposits of

fibrin, up to a few millimeters in thickness, were found in the mid-portions of the graft as late as 6 months following operation. Two grafts were completely thrombosed in their mid-portions, 2 and 4 months following operation. In these animals there was microscopic or gross evidence of infection.

Fig. 7.—Microscopic section of stapled aorta-Dacron anastomosis.

A, Specimen shows everted cuff of graft *(right)* within lumen of aorta. Note ridge formed by cuff of graft. The surface facing the lumen of the aorta is at the top. (Elastic tissue stain; ×4.5.)

B, Close-up of same specimen. Compression of elastic fibers by staple is well seen. There is no inflammatory reaction and no other medial injury. (Elastic tissue stain; ×30.)

DISCUSSION

The earliest report of a vascular stapling apparatus, by Gudov, 10 years ago, is in Russian and was not available to us. This is apparently the precursor of the device described more recently by Androsov,[1] which employs tantalum wire staples and is a complex, precision instrument of approximately 26 parts. It is said to be usable for suturing vessels from 1.3 to 15 mm. in diameter. Eversion of the ends of both vessels being joined is required. The instrument

was reported to have been tested in 394 experimental operations on dogs with excellent results; there was no narrowing at the anastomotic site, no thrombosis, no scar formation, and no inflammatory reaction around the clips. Details of these experiments were not given.

A Hungarian stapling apparatus was described in 1953 by Bikfalvi and Dubecz[2] along with details of its use in 47 animals. Stapling of the abdominal aorta with this device was successfully accomplished in 27 animals, of which there were 18 survivors. The details of construction of the device are not easy to make out. Suturing was accomplished by U-shaped silver clamps.

The third report of a blood vessel stapler to appear was that of Vogelfanger and Beattie from Canada.[3] Again, the finer details of the mechanics of the instrument are not clear, but the construction seems to be considerably simpler than that of the preceding two. Tantalum U-shaped staples were used in the abdominal aorta of 10 pigs. Few details of the experimental work done were given in this report.

The description of a stapling device of Japanese construction, by Inokuchi, next appeared.[4] This resembles the Russian stapler but is of simpler design. Alloyed steel clips were used in numerous animal experiments. Again, few details were reported. However, in a personal communication, Dr. Inokuchi[8] kindly sent details of the results of experiments in 68 animals, comparing suturing techniques with stapling techniques in vessels ranging from 2.6 mm. to 9.0 mm. in size. There was clear-cut superiority of the stapling technique over suturing by hand using the Carrel technique, in both veins and arteries.

The fifth stapling device of which we have knowledge is of a totally different design and was reported by Sterling of Philadelphia.[5] This instrument does not place all the staples into the anastomosis simultaneously as the others do, but instead, singly, by a device which rotates the anastomosis through the stapler. No experimental or clinical applications were described.

Our instrument, the sixth to appear, has the virtue of simplicity of design since it consists of but eight parts. It has functioned successfully over a fairly broad range of vessel sizes. Staples have been made of both stainless steel and of a gold alloy.

The method of anastomosing graft to vessel by intubation of the vessel with the everted graft seems attractive because the vessel wall need not be traumatized by instruments nor everted on the coupling device. It seems to be considerably easier technically to intubate a vessel than it is to evert the cut end of a vessel over a device, especially if the vessel wall is thick or stiff because of disease. The technical excellence of some anastomoses, the metallic, nonreactive nature of the staples, and their minimal contact with the blood stream also recommend stapling methods generally. Speed of effecting the union is another advantage.

On the other hand, the need for a special device of the correct size, smaller than the vessel to be anastomosed, may prove to be a serious disadvantage when working with vessels of small caliber. The collections of fibrin in the mid-portion of some grafts may be taken as evidence of the effects of turbulence due to nar-

rowing at the anastomotic junction in these animals. The thickness of the graft material (the everted cuff was 2 mm. in thickness) and the relative rigidity of the thick-walled thoracic aorta undoubtedly exaggerated the degree of narrowing which this technique produces. It is possible that with thin-walled veins as grafts to small arteries the degree of narrowing at the anastomosis may be insignificant. The report by Androsov and the experimental data provided by Inokuchi support this view. Our own experience with the use of this stapler in end-to-end anastomosis of the divided superior vena cava in animals also encourages us to expect better results with thin-walled vessels and prostheses.

Another disadvantage of this technique is the need for graft material of sufficient elasticity to prevent wrinkling when the graft is everted upon the stapler. Braided nylon, elasticized "Fluflon" Dacron, and homografts, as well as autografts, have all been used successfully in such devices.

Excessive bleeding between staples may be overcome by diminishing the distance between staples or by adding a second row of staples, staggered with respect to the first row.

Cuffing of the blood vessel over the graft makes it necessary to free up longer segments of blood vessel than the ordinary suturing techniques require. It is possible, however, that the advantages of obtaining technically excellent anastomoses in small vessels, or speedy anastomoses in large vessels with multiple branches (aortic arch, thoracoabdominal aorta), by mechanical means may outweigh the disadvantages of the additional dissection required to provide adequate cuffs of vessels.

SUMMARY AND CONCLUSIONS

1. A simple device, of but eight parts, for anastomosing a prosthetic graft to the canine thoracic aorta by intubating the aorta with the everted graft to achieve union is presented. Stapling is accomplished while blood is flowing through the device.

2. The instrument was used to interpose an elasticized Dacron graft in the descending thoracic aorta of 25 animals. There were 2 operative deaths, and 2 late deaths because of infection, with thrombosis of the graft. The remaining animals had patent grafts with anastomotic junctions which were technically excellent for the most part.

3. Significant narrowing at the anastomotic junction, which was due in part to disparity between the aorta and the graft and in part to cuffing of the vessel over the graft and of the graft upon itself, was observed in some animals. This may be overcome if prostheses sufficiently thin and elastic can be produced to allow temporary distortion by the stapling device, until stapling can take place. Vein grafts would probably answer the purpose.

4. A smaller model of the instrument, suitable for use in vessels 3 mm. in diameter, is being constructed. It is in the area of small vessel anastomoses that such devices may find their greatest usefulness.

We wish to acknowledge with gratitude the very real contribution of Mr. A. W. Chattaway of the Asheville Tool and Gauge Company in helping to plan this stapling device and in constructing the initial model.

REFERENCES

1. Androsov, P. I.: New Method of Surgical Treatment of Blood Vessel Lesions, A. M. A. Arch. Surg. **73**: 902-910, 1956.
2. Bikfalvi, A., and Dubecz, S.: Observations in Animal Experiments With Mechanized Vessel Suture, J. internat. chir. **13**: 481-496, 1953.
3. Vogelfanger, I. J., and Beattie, W. G.: A Concept of Automation in Vascular Surgery: A Preliminary Report on a Mechanical Instrument for Arterial Anastomosis, Canad. J. Surg. **1**: 262-265, 1958.
4. Inokuchi, K.: A New Type of Vessel Suturing Apparatus, A. M. A. Arch. Surg. **77**: 954-957, 1958.
5. Sterling, J. A.: Stapling Small Blood Vessels, J. Albert Einstein Med. Center **7**: 192-193, 1959.
6. Takaro, T., and Crymes, T. P.: Reconstruction of the Thoracic Aorta With the Aid of a Vascular Coupling Device (Vessel Couple). Report of a Case, J. THORACIC SURG. **37**: 93-99, 1959.
7. Takaro, T.: Experimental Replacement of Segments of the Upper Thoracic Aorta With the Aid of Vessel Couples, Am. J. Surg. **98**: 617-622, 1959.
8. Inokuchi, K.: Personal communication, 1960.

(For Discussion, see page 699)

EXPERIMENTAL LEFT CORONARY ARTERY PERFUSION THROUGH AN AORTOTOMY DURING CARDIOPULMONARY BYPASS

James B. Littlefield, M.D. (by invitation), Edward M. Lowicki, M.D. (by invitation), and William H. Muller, Jr., M.D., Charlottesville, Va.

A DIRECT surgical approach on the aortic valve often necessitates a prolonged aortotomy, during which time the maintenance of myocardial nutrition is mandatory. The desirability of utilizing coronary artery perfusion to maintain nutrition in the bypassed heart with an ascending aortotomy is now well established. Clinically, we have found left coronary artery perfusion to be very satisfactory and the approach to the aortic valve is facilitated with a single cannula in the operative field.[1] The difficulties associated with retrograde coronary perfusion have been presented by Shumway[2] and Melrose.[3]

Our interest in experimental coronary artery perfusion began 2 years ago during the laboratory investigation of prosthetic aortic valve replacement. It was immediately apparent that the lack of a suitable cannula tip to accommodate the coronary ostia of the dog limited successful coronary artery perfusion through an open aortotomy. Other investigators have reported similar experiences.[2, 4] Fig. 1 shows several cannulas which were studied before the tip (Fig. 1, *e*) now used experimentally for left coronary artery perfusion was developed. The multiple holes present are necessary to avoid obstruction of the blood flow to early branches of the left coronary artery.

EXPERIMENTAL METHOD

Sixty-one dogs (10.9 to 18 kilograms) were studied, 15 as controls, 24 in acute experiments, and 22 as survivor animals. Sodium pentobarbital anesthesia was used and positive endotracheal pressure (9 to 12 cm./water) was maintained by a mechanical respirator. A right thoracotomy through the fourth intercostal space and complete pump dependency (utilizing a disc oxygenator) were employed. The ascending aorta was cross-clamped within the pericardium and a 2 cm. oblique incision was made just above the aortic valve. The 15 control animals were studied by allowing blood from the left ventricle to flow freely through the open aortotomy, where it was removed by suction until either ventricular fibrillation or cardiac arrest occurred. In the remainder of the animals, a single coronary perfusion cannula, connected either to the arterial line of the pump or to a reservoir (500 ml. bottle)

From the Department of Surgery, University of Virginia School of Medicine, Charlottesville, Va.

Supported by U. S. Public Health Service Grants H-2038 and H-4642.

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

suspended 100 cm. to 110 cm. above the heart, was inserted into the left coronary ostium. Blood flow from the left ventricle was aspirated through the aortotomy. The right coronary ostium was not perfused. The suspended reservoir maintained a constant pressure of 74 to 80 mm. Hg[5] and continuous perfusion was performed for periods of 15 to 60 minutes at temperatures between 28° and 36° C. (esophageal and rectal). Coronary artery flow rates measured prior to perfusion varied from 50 ml. to 150 ml. per minute. A cannula was placed into the right auricle in 17 of the animals studied and total coronary venous flows (coronary sinus plus anterior cardiac venous return) were measured with a graduate cylinder; coronary venous samples for oxygen determination were drawn at intervals. Distal aortic pressures, continuous electrocardiographic tracings, coronary venous flows, temperatures, and coronary arteriovenous oxygen saturations (determined photoelectrically) were recorded. At the completion of coronary artery perfusion, the aortotomy was closed, the aortic clamp released, and pump dependency discontinued as soon as the animal's temperature reached 34° C. A section of Gelfoam saturated in protamine was placed over the aortotomy immediately after closure to produce local clotting until protamine sulfate had been administered systemically. Postoperative electrocardiograms were taken at intervals in the survivor animals. Digitalis was used in only 2 animals that died within a few hours after operation.

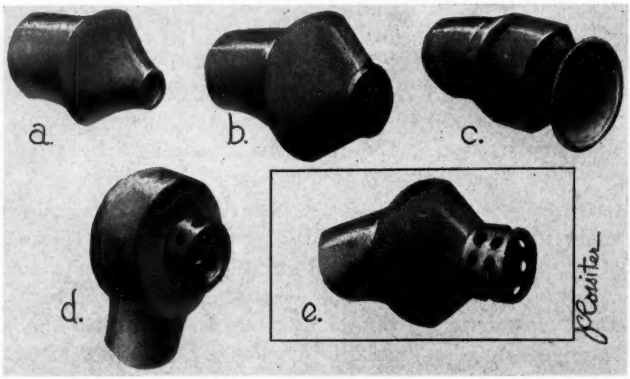


Fig. 1.—These metal cannula tips were studied during this investigation in developing tip *e*, now in use for left coronary artery perfusion in the experimental animal. Tips *a*, and *c*, were designed to fit within the coronary ostium and did not have to be held in place. Tip *e*, may be used on either a malleable or firm metal cannula; it is 5 mm. in length with an internal diameter of 3.5 mm.

RESULTS

The success of this investigation was limited by several factors, (1) the need for a specially designed cannula tip to accommodate the dog's left coronary ostium, (2) anatomic variations of the left coronary artery characterized by an immediate bifurcation, trifurcation, or double ostium, (3) the difficulty in maintaining proper alignment of the coronary cannula during prolonged periods of perfusion, (4) the dog's small, friable, ascending aorta, and (5) complications associated with extracorporeal circulation.

Control Studies.—In the 15 control animals studied (Table I) ventricular fibrillation or cardiac arrest occurred within 8 to 45 minutes after opening the aorta. The electrocardiogram in 5 of these dogs showed regular electrical activity (without ventricular fibrillation) after 60 minutes, even though the heart

visibly was in arrest. Coronary artery perfusion was not performed at any time in the control group. Through the open aortotomy of the control animals, we observed that there was always a small, slow flow of blood running from both coronary ostia into the aorta, irrespective of cardiac rhythm. We have made this same observation during other investigations, while employing cardiac arrest and an open aortotomy, in the empty, bypassed heart.

TABLE I. CARDIAC RESPONSE OF 15 CONTROL ANIMALS TO AN OPEN AORTOTOMY WITHOUT CORONARY PERFUSION DURING BYPASS

MEAN TEMPERATURE	ANIMALS STUDIED	MEAN PERIOD BETWEEN OPENING AORTA AND ONSET OF CARDIAC ARREST OR VENTRICULAR FIBRILLATION
31°-33° C.	5	26.0 minutes
33°-35° C.	5	9.2 minutes
35°-37° C.	5	26.0 minutes

Acute Studies.—These 24 experiments were terminated acutely because of technical difficulties encountered during the procedures. Excessive aortotomy bleeding, anatomic variations of the left coronary artery, high plasma hemoglobin, unsuitable coronary cannula tips, and complications of extracorporeal circulation were the major problems in this group. Fifteen of these dogs were studied during the first 25 experiments while a suitable cannula tip was being developed and the operative procedure standardized.

Survivor Studies.—Twenty-two dogs survived from 3 hours to 3½ months (Table II). The early deaths, within 9 days, resulted from: generalized bleeding secondary to cardiopulmonary bypass or postoperative hemorrhage from the aortotomy; sudden death of undetermined cause; infection; cardiac failure; myocardial infarction; and a high plasma hemoglobin (270 to 400 mg. per cent). There was one late death due to empyema.

Continuous perfusion of the left coronary artery was performed, without ventricular fibrillation, for periods of 15 to 60 minutes; the cardiac rate during perfusion varied between 60 and 90. Flows from the coronary cannula (50 ml. to 150 ml. per minute) were measured prior to perfusion but angulation of the cannula plus coronary vascular resistance reduced these initially determined flows. We found the most accurate determination of coronary perfusion was obtained by measuring the total coronary venous return from the right auricle. Total coronary venous flow in the nonworking, bypassed heart, before left coronary artery perfusion, averaged 53 ml. per minute; flows from 40 ml. to 50 ml. per minute proved adequate during perfusion. The suspended reservoir filled with oxygenated blood from the pump permitted coronary perfusion at a constant pressure of 74 to 80 mm. Hg; this was technically preferable to direct perfusion from the arterial line of the pump, although both methods were used successfully in this study.

Results of the mean coronary arteriovenous oxygen differences, expressed in per cent saturation, are summarized in Fig. 2. The bypassed, nonworking heart shows a value of 37.5 per cent which falls to 24.3 per cent (sinus rhythm) and

TABLE II. RESULTS OF THE 22 ANIMALS STUDIED IN SURVIVOR EXPERIMENTS WITH LEFT CORONARY ARTERY PERFUSION ALONE

PERIOD OF SURVIVAL	NUMBER OF ANIMALS	DURATION OF LEFT CORONARY ARTERY PERFUSION (MIN.)	CARDIAC RHYTHM DURING PERFUSION		COMMENTS
			SINUS	VENT. FIB.	
					Cause of death
12 hours or less	4	60	x		Postoperative hemorrhage (aortotomy)
		60	x		Right heart failure
		20	x		Cause undetermined
		17	x		High plasma hemoglobin (270 mg. %)
12 to 24 hours	6	18	x		High plasma hemoglobin (400 mg. %)
		30		x	Right heart failure*
		33		x	Postoperative hemorrhage
		37		x	Right heart failure*
		45		x	Postoperative hemorrhage
		45	x		Postoperative hemorrhage
1 to 9 days	7	15	x		High plasma hemoglobin (400 mg. %)
		24	x		Infarction of right ventricle
		25		x	Empyema (9th day)
		30	x		Complication of pump-oxygenator
		30	x		Postoperative hemorrhage
		47		x	Postoperative hemorrhage (aortotomy)
		48		x	Postoperative hemorrhage
6 weeks or longer	5	18	x		Sacrificed at 8 weeks
		20	x		Sacrificed at 6 weeks
		20	x		Sacrificed at 14 weeks
		21	x		Sacrificed at 8 weeks
		23	x		Empyema (6 weeks)

*One dog showed immediate bifurcation and the other immediate trifurcation of the left coronary artery

24.7 per cent (ventricular fibrillation) during perfusion of the left coronary artery. After unclamping the aorta there is an additional fall to 21.7 per cent in the bypassed heart. The mean oxygen consumption of the entire heart during left coronary artery perfusion was 2.54 ml. per 100 Gm. heart weight per minute in normal sinus rhythm and 2.74 ml. per 100 Gm. heart weight per minute with ventricular fibrillation. In Fig. 2 the results of this investigation are compared with the experimental coronary perfusion studies of others in the totally bypassed, nonworking heart[6] and in the nonworking heart separated from the body as an isolated preparation.[7] The results under these three conditions are essentially the same during adequate coronary perfusion.

Electrocardiographic studies in this investigation demonstrated that when coronary perfusion was inadequate, during the open aortotomy, evidence of myocardial ischemia was present throughout the perfusion period and often persisted until the death of the animal. When adequate coronary perfusion was maintained, however, even during periods of ventricular fibrillation with the aorta open, electrical defibrillation was usually immediate and prompt cardiac recovery followed. None of the long-term survivor animals developed ventricular fibrillation or other significant arrhythmias during their investigation.

DISCUSSION

This study demonstrates several important factors which limit successful left coronary artery perfusion in the experimental animal, and the need for utilizing a special cannula tip to accommodate the dog's left coronary artery.

Adequate left coronary artery perfusion was performed from a suspended reservoir which maintained a pressure above 74 mm. Hg. Jardetzky and associates[5] have shown that a constant perfusion pressure of 65 mm. Hg furnishes an adequate coronary flow, with an unaltered oxygen consumption for 3 hours, in the isolated, empty, nonworking, dog's heart with sinus rhythm.

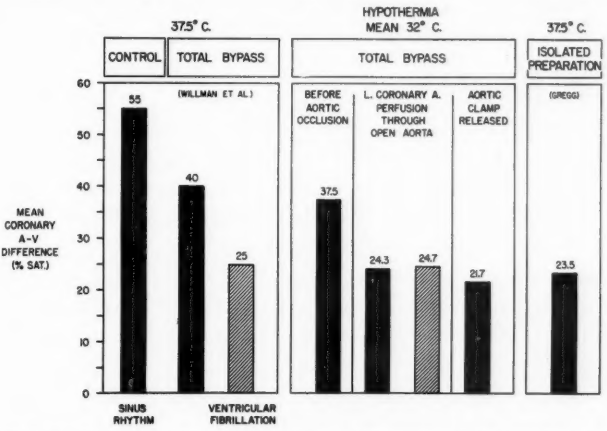


Fig. 2.—Coronary arteriovenous oxygen differences of this study are shown in the *middle* section and are compared with results from the investigation of Willman et al.[6] (totally bypassed closed heart) and studies summarized by Gregg[7] (isolated heart preparation). All are studies in the dog heart. Normal sinus rhythm is shown in solid black and ventricular fibrillation by cross-hatching.

Continuous left coronary perfusion *alone* was performed from 15 to 60 minutes in this investigation without fibrillation. Shumway[2] has reported similar results during 15 minutes of left coronary artery perfusion. We found during prolonged periods of ventricular fibrillation, with adequate left coronary artery perfusion, that electrical defibrillation was immediate and cardiac recovery prompt. These findings have been substantiated clinically and apparently ventricular fibrillation during an open aortotomy is not harmful, providing the coronary vascular bed is *adequately perfused.*

The mean coronary arteriovenous oxygen differences (using animals of about equal size), during adequate left coronary artery perfusion at an average temperature of 32° C., were the same in the presence of ventricular fibrillation or a normal sinus rhythm (Fig. 2). Willman, Neville, and Hanlon[6] reported similar values in the normothermic, nonworking, bypassed, dog's heart, without an aortotomy; both coronary arteries were perfused normally in their investigation. Gregg's[7] summary of studies in the normothermic, isolated, nonworking, canine heart with sinus rhythm showed the coronary arteriovenous oxygen difference to be approximately the same as the open, nonworking, left coronary perfused hearts during sinus rhythm of this investigation (Fig. 2).

The myocardial oxygen consumption during left coronary artery perfusion of the nonworking, open heart, in this experimental study, was the same with fibrillation or normal sinus rhythm. Beuren, Sparks, and Bing[8] found no statistical difference, in the closed chest dog, between the oxygen consumption of the artificially perfused, empty heart during sinus rhythm or ventricular fibrillation.

SUMMARY AND CONCLUSIONS

1. Continuous left coronary artery perfusion alone may be performed for prolonged periods through an ascending aortotomy without ventricular fibrillation. This procedure is frequently limited by the anatomic variations of the dog's left coronary artery.

2. Arterial perfusion which provided a total coronary venous return of 40 ml. to 50 ml. per minute was adequate in the nonworking heart with an ascending aortotomy.

3. Coronary artery perfusion from a suspended reservoir at a constant pressure of 74 to 80 mm. Hg was preferable to arterial perfusion from the pump, although both methods were used successfully.

4. Coronary arteriovenous oxygen differences and oxygen consumption in the nonworking dog's heart, during left coronary perfusion through an aortotomy, were approximately the same with normal sinus rhythm and ventricular fibrillation.

5. The long-term survivor animals did not develop fibrillation during their investigation. However, in other dogs, when adequate coronary artery perfusion was maintained during periods of ventricular fibrillation, electrical defibrillation was immediate and cardiac recovery prompt.

6. The most important single factor contributing to cardiac recovery during an open aortotomy in this investigation was *continuous adequate coronary perfusion,* irrespective of variations in temperature, cardiac rhythm, and the method or duration of perfusion.

7. Clinically left coronary artery perfusion, performed from the arterial line of the pump through an ascending aortotomy, has proved very satisfactory regardless of the duration of perfusion. In the experimental animal, prolonged left coronary artery perfusion may be a complicated procedure and is difficult to execute consistently with success.

We wish to express our appreciation to Mr. Thomas Perry, Mr. Eugene Varga, Mrs. Mary E. Garrard, and Mr. Boyd Perry for their technical assistance.

REFERENCES

1. Muller, W. H., Jr., Warren, W. D., Dammann, J. F., Jr., Beckwith, J. R., and Wood, J. E., Jr.: Surgical Relief of Aortic Insufficiency by Direct Operation on the Aortic Valve, Circulation 21: 587, 1960.
2. Shumway, N. E.: Forward Versus Retrograde Coronary Perfusion for Direct Vision Surgery of Acquired Aortic Valvular Disease, J. THORACIC SURG. 38: 75, 1959.

3. Melrose, D. G.: Discussion *in* Extracorporeal Circulation, Springfield, 1958, Charles C Thomas, publisher, p. 498.
4. Headrick, J. R., Strayhorn, W. D., Munoz, A. J., and Adams, J. E.: A Method of Safely Prolonging Induced Cardioplegia, S. Forum **10**: 509, 1959.
5. Jardetzky, O., Greene, E. A., and Lorber, V.: Oxygen Consumption of the Completely Isolated Dog Heart in Fibrillation, Circulation Res. **4**: 144, 1956.
6. Willman, V. L., Neville, E. C., and Hanlon, C. R.: Cardiac Metabolism Under Conditions Associated With Open Heart Operations. I. Coronary Sinus Flow and Myocardial Oxygen Consumption, S. Forum **8**: 287, 1957.
7. Gregg, D. E.: Coronary Circulation in Health and Disease, Philadelphia, 1950, Lea & Febiger, p. 154.
8. Beuren, A., Sparks, C., and Bing, R. J.: Metabolic Studies on the Arrested and Fibrillating Perfused Heart, Am. J. Cardiol. **1**: 103, 1958.

(For Discussion, see page 699)

THE GROWTH OF CARDIOVASCULAR CHAMBERS FOLLOWING CARDIAC AND AORTIC SURGERY

Sigmund A. Wesolowski, M.D. (by invitation), Lester R. Sauvage, M.D. (by invitation), Philip N. Sawyer, M.D. (by invitation),** Karl E. Karlson, M.D., and Lester Fox, M.D. (by invitation), Brooklyn, N. Y., and Boston, Mass.*

THE DEVELOPMENT of intracardiac surgery for the correction of human congenital cardiac defects has been so rapid as to outstrip the available knowledge from the experimental laboratory concerning the long-term fates of the intracardiac procedures performed. This communication presents the results of our initial characterization of the growing pig preparation which we feel gives equivalent long-term results very rapidly. In this preparation all cardiac and vascular diameters double in a 6-month period of maturation, "degenerative" calcific changes occur in or around implants during this 6-month period, and this preparation has proved to be a very sensitive indicator of aberrations in the outflow tracts of the ventricles and great vessels.

METHOD

Left Ventricular Studies.—The present report includes 209 pigs bearing 5.5 cm. long, isodiametric grafts of various materials which replaced resectional defects of the descending thoracic aorta. These grafts were implanted at pig body weights of from 14 to 44 pounds (10 to 12 weeks of age). Pigs were allowed to mature for 6 to 12 months to be slaughtered at an average body weight of 235 pounds. At slaughter, the heart and thoracic aorta were removed from each animal; the aorta containing the graft was x-rayed after inflation with air at 120 mm. Hg pressure. Details of the fates of these grafts will be published elsewhere.[5] Measurements of internal diameters were taken from the roentgenograms: graft diameter used was the minimum internal diameter while the distal aortic diameter used was the largest diameter between the distal end of the graft and the first patent intercostal artery. In the stenotic grafts, poststenotic dilatation was corrected for as shown in Fig. 1. The hearts of these

From the Department of Surgery, State University of New York Downstate Medical Center and Kings County Hospital, Brooklyn, the Department of Surgery, Children's Hospital, Boston, and the Department of Pathology, St. John's Episcopal Hospital.

This work was supported in part by U. S. Public Health Grants H-3286, H-3286(S1), and H-3286(C1).

Read at the Fortieth Annual Meeting of The American Association for Thoracic Surgery at Miami Beach, Fla., May 11-13, 1960.

*Third Research Scholar, American College of Surgeons.

**Markle Scholar in Medical Science.

same animals were trimmed in the fresh state as follows: the right ventricle was cleanly excised from the atrium at the tricuspid valve ring and from the ventricular septum and included that portion of the pulmonary artery bearing the sinuses of the pulmonary valves; the left ventricle included the ventricular septum but excluded the entire aorta and atrium at the aortic and mitral valve rings, respectively. The cavities and muscular trabeculae were cleaned of all thrombi and each ventricle separately weighed. Good reproducibility of this ventricular trim technique in our hands is demonstrated in Table I and has independently been reported by Busch[2] as a valid technique for studying the human heart.

TABLE I. VENTRICULAR TRIM—NORMAL ADULT PIG HEART

OBSERVER	NUMBER OF HEARTS	MEAN RV/LV	STANDARD DEVIATION
S. A. W.*	6	33.5%	± 1.8%
S. A. W.*	19	31.9%	± 1.8%
K. E. K.**	12	33.7%	± 1.6%
A. L. S.***	11	34.0%	± 2.0%

*Trimmed all hearts in remainder of study.
**Author.
***Graduate Nurse Research Assistant.

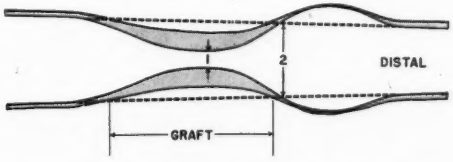


Fig. 1.—Diagram of the method for correction of diameter relationship in the presence of post-stenotic dilatation. The dotted lines represent the normal aortic taper in the absence of stenosis or post-stenotic dilatation. Corrected percentile patency, therefore, is equal to diameter at 1 / diameter at 2 ×100 per cent.

Right Ventricular Studies.—This report also includes 11 other pigs in which one of four different right ventricular outflow tract aberrations was created at the standard pediatric age: 2 pigs had simple resection of one pulmonic valve cusp using a partial occlusion technique; 2 pigs had simple elliptical excision of a portion of the infundibulum, one pulmonic valve cusp and sinus with simple closure and bicuspidization of the pulmonic valve using a partial occlusion technique—the average systolic gradient produced by this degree of stenosis at operation was found to be 8 mm. Hg; 2 animals had simple elliptical excision as above and replacement of the excisional defect with an isometric Ivalon patch of 8:1 compression ratio; the remaining 5 animals had elliptical excision with isometric replacement of the excisional defect with autologous fibrous pericardium. Patch replacements were accomplished using moderate depth immersion hypothermia and inflow-occlusion for 3 to 5 minutes; these patches averaged 2.7 by 2.0 cm. at implantation. These animals were allowed to mature (as above). At slaughter, the heart was removed, x-rayed in the anteroposterior and lateral projections with the pulmonary artery and right ventricle inflated with air at a pressure of 30 mm. Hg. The ventricles were individually trimmed and weighed as above.

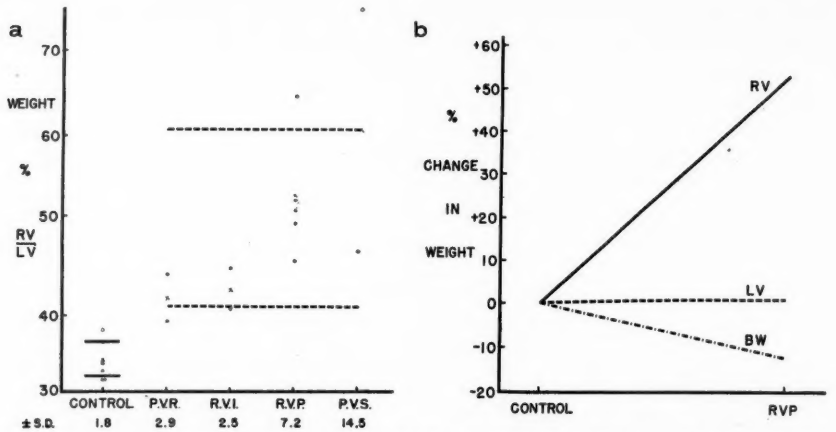


Fig. 2.—*a*, Graph which plots percentile RV/LV weight ratios for various right ventricular outflow tract aberrations. Controls are 6 pigs with no cardiovascular lesions. *P.V.R.* = 1 cusp pulmonic valve resection. *R.V.I.* = Ivalon patch in right ventricular outflow tract. *R.V.P.* = free autologous pericardial patch in right ventricular outflow tract. *P.V.S.* = elliptical excision of one cusp of pulmonic valve with bicuspidization of remaining valve. The solid black lines in the controls represent values of ±1 S.D. whereas the dotted lines represent values of ±1 S.D. for the entire group of pigs with right ventricular outflow tract aberrations. Note that there is no overlap of the two sets of standard deviations.

b, Graph which demonstrates the percentile increases over control values of the weights of the right ventricle, left ventricle, and total body in pigs bearing free pericardial patches replacing elliptical excisions of one pulmonic-valve cusp. All control and RVP pigs were mature at the time of slaughter; the pericardial patches had been inserted at 12 weeks of age.

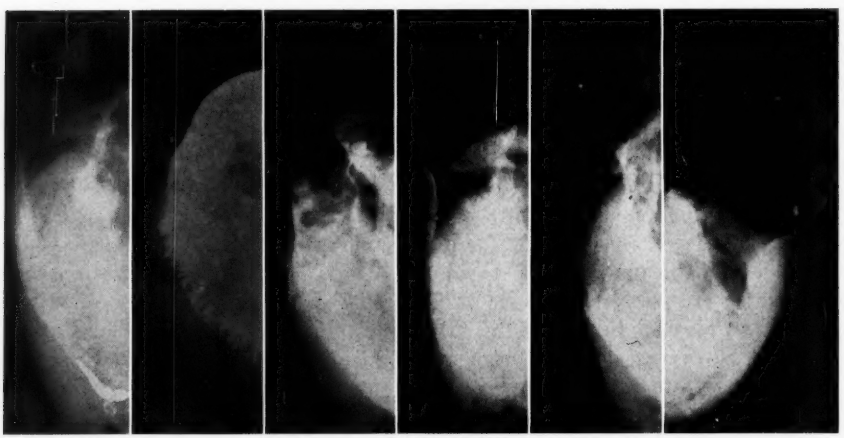


Fig. 3.—Lateral roentgenograms of the right ventricular outflow tract and main pulmonary artery of the hearts of pigs. All pigs were operated upon at about 30 pounds of weight and all were mature (200 pounds or more) except in *B* (76 pounds) at death or sacrifice. Tract in *B* filled with Diodrast; all others inflated with air at 30 mm. Hg pressure. *A*, Normal adult pig. *B*, Resection 1 cusp pulmonic valve; note increased trabeculation of right ventricle. *C*, Elliptical excision of 1 cusp with bicuspidization of the pulmonic valve; note preservation of same degree of stenosis with growth and post-stenotic dilatation of pulmonary artery here and in *D*. *D*, Elliptical excision with Ivalon patch replacement with development of slight stenosis with growth because of the inability of growth of the Ivalon patch; note also calcification of the Ivalon patch verified by histologic examination. *E*, Elliptical excision with free pericardial patch replacement with preservation of relatively normal diameter relationships with growth *F*, same as *E* but with aneurysmal dilatation of pericardial patch.

RESULTS AND CONCLUSIONS

There is a gross correlation between age and ventricular weights, but the ratio of the individual weights of the ventricles (RV/LV) of all animals beyond 20 weeks of life was found to fall within the normal adult ratios listed in Table I. The RV/LV weight ratio was used, therefore, as the cardiac parameter to be studied.

Right Ventricular Studies.—In all 11 animals with minimal right ventricular outflow tract aberrations there was a significant increase in the RV/LV weight ratio as summarized in Fig. 2, *a*; this increase was due to increase in the RV weight over control values as summarized in Fig. 2, *b* which also demonstrates that the ventricular changes are independent of body weight. Eight of these animals and the 6 control animals with no outflow tract aberrations were

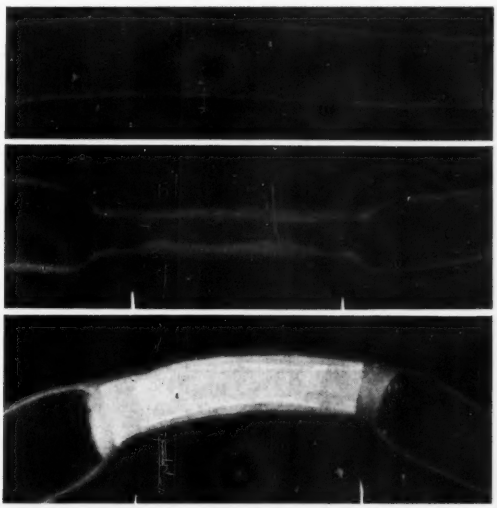


Fig. 4.—Roentgenograms of examples of graft stenosis at coarctation site. Specimens inflated with air at 120 mm. Hg pressure. *Upper*, aortic autograft with 100 per cent patency (0 per cent stenosis). *Middle*, prosthetic graft with 50 per cent patency. *Lower*, prosthetic graft with complete occlusion or 0 per cent patency. Note the calcific changes within the latter prosthetic graft.

slaughtered between 5 to 6 months after operation. Three animals died as follows: 1 with pulmonic valve regurgitation died of pneumonia 118 days after operation, another with the same lesion died of large bowel obstruction caused by rectal prolapse at 134 days, and the third, with stenosis, died *with congestive heart failure* 36 days after operation. The gross changes in the right ventricular outflow tract are summarized in Fig. 3. Space does not permit the detailed description of these changes but suffice it to say that pulmonary valve stenosis or regurgitation of a degree considered to be clinically insignificant produces ventricular changes easily detectable in the preparation of the growing pig within a 6-month period of maturation.

Left Ventricular Studies.—In the 209 animals implanted with various kinds of isodiametric grafts to replace defects in the upper descending thoracic aorta, varying degrees of graft stenosis were observed to occur consequent to maturation. Examples of graft stenoses are shown in Fig. 4. The RV/LV weight ratio was proportionately lowered at graft diameters less than 50 per cent of the average adult pig aortic diameter at the standard graft site (Fig. 5). When the degree of stenosis was corrected for varying aortic diameter (and also for poststenotic dilatation as shown in Fig. 1), the RV/LV weight ratio is shown to decrease at *all* degrees of stenosis, even at high patency values (graft diameter/distal aortic diameter), as demonstrated in Fig. 6, *a*. That the lowering of the RV/LV weight ratio is due to increase in the left ventricular weight below 80 per cent graft patency values is demonstrated in Fig. 6, *b*. Indeed, below 60 per cent patency there is a smaller and concomitantly progressive increase in the right ventricular weight. This rise in right ventricular weight is of a

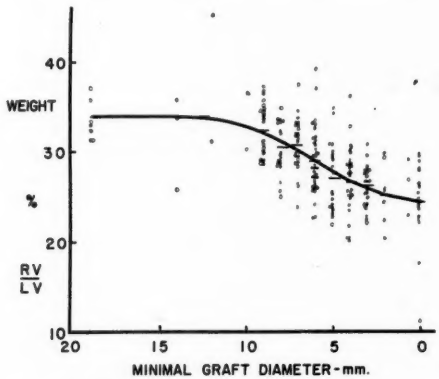


Fig. 5.—Graph which plots percentile weight RV/LV against minimal graft diameter. From simple observation of these data it would seem that there is no aberration of the RV/LV weight ratio until the stenosis is less than 10 mm. which apparently is about 50 per cent of the normal aortic diameter at this site.

lower order of magnitude than the left ventricular rise and may well be due to an elevated end diastolic left ventricular pressure (and therefore elevated pulmonary circuit pressure) consequent to left ventricular hypertrophy. It is sufficient to the purposes of this communication, however, to point out that there are easily detectable effects upon the heart of even mild degrees of stenosis of the outflow tract of the left ventricle even at some distance removed from the aortic valve, such as in the upper descending thoracic aorta. These changes with growth of the animal can be detected within a 6-month period of observation.

DISCUSSION

What is the significance of aneurysm formation of autologous pericardium implanted into the outflow tract of the right ventricle? In the pig the fibrous pericardium is much more flimsy than in the dog and human being. This is

demonstrated by the fact that free autologous pericardial implants used to replace thoracic aortic defects in the growing pig[5] rupture within a week after operation, whereas those in the adult dog[4] remain intact for periods of 18 months or more without rupture or aneurysm formation.

What is the significance of ventricular hypertrophy? Certainly lesions that produce marked degrees of ventricular hypertrophy can be lethal and it would seem reasonable to assume that minimal degrees of hypertrophy are innocuous. Moderate degrees of ventricular hypertrophy, as found in this study, are well tolerated on short-term follow-up but we have no data as to the relationship of

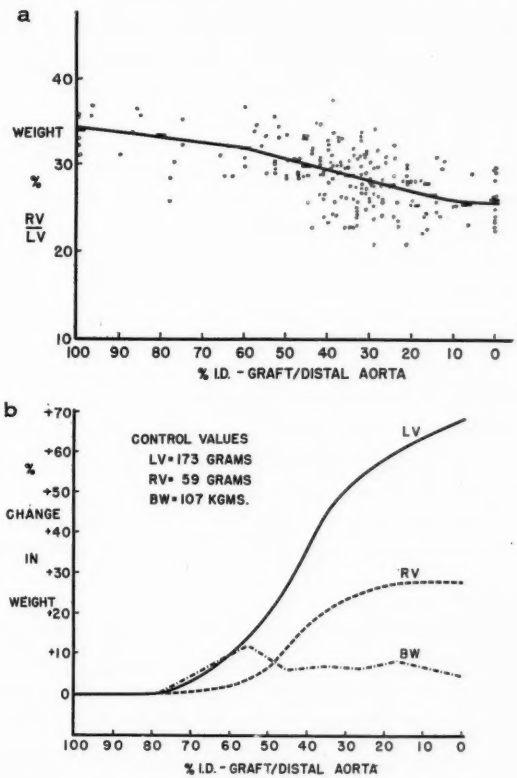


Fig. 6.—*a*, Graph which plots percentile weight RV/LV against percentile patency which corrects for varying aortic diameter distal to the site of stenosis. With such correction, even mild degrees of stenosis in the coarctation site are detected by the RV/LV weight ratio. *b*, Graph which plots percentile deviation from control values of ventricular weights against percentile patency of grafts at the coarctation site. Note that the left ventricular weight increases at patency values below 80 per cent and that a smaller percentile increase in right ventricular weight occurs at patency values below 60 per cent.

moderate ventricular hypertrophy to longevity or to increased susceptibility to angina pectoris on long-term follow-up. Without entering into a discussion of surgical philosophy, the procedure which leads to less ventricular hypertrophy would be the preferable. It is suggested that choice of intracardiac procedure

could be accurately and rapidly screened in the growing pig preparation and, thus, accelerate the progress of intracardiac surgery.

What is the significance of calcification of Ivalon implants into the heart? The report of Blumberg, Griffith, and Merendino[1] that Ivalon implants in the human can calcify, become rigid, and erode an aortic valve leaflet demonstrates that calcification of a prosthetic may be undesirable. The present communication demonstrates that the preparation of the growing pig can detect calcific deposit in prosthetic cardiac implants within a period of 6 months.

SUMMARY

1. Lesions simulating clinically minimal pulmonic stenosis, pulmonary valve regurgitation, and right ventricular outflow tract widening, using patches of Ivalon or autologous fibrous pericardium, have been produced in growing pigs. The pigs were allowed to mature.

2. In a large series of growing pigs, defects of the thoracic aorta were bridged, using various graft materials. At maturity these grafts were found to be variously stenosed.

3. The right ventricular weight and RV/LV weight ratio were found to be moderately elevated over control values in all of the mature pigs bearing minimal right ventricular outflow tract aberrations.

4. Ivalon implants in the outflow tract of the right ventricle produced mild stenosis with growth and attracted calcific deposit while free autologous fibrous pericardial implants formed aneurysms.

5. Stenosis at the thoracic aortic coarctation site produced increase in left ventricular weight and decrease in the RV/LV weight ratio when compared to control values. These changes were observed when the stenosis was greater than 20 per cent. (Less than 80 per cent patency.) Lesser increases in right ventricular weight were also observed at diameter ratios less than 60 per cent; the latter increases did not correlate with differences in age or body weight of the animals.

6. It is concluded that the growing pig can be used as a sensitive preparation for the rapid screening of the comparative hemodynamic effectiveness and expected clinical worth of cardiac and thoracic aortic surgical procedures. It is further suggested that, in addition to such screening, certain other species-transferable data, such as calcification of cardiovascular implants, can be concomitantly gained.

REFERENCES

1. Blumberg, Y. B., Griffith, P. C., and Merendino, K. A.: The Effect of Specific Compression on Soft-tissue Response to Formalinized Polyvinyl Alcohol (Ivalon) Sponge, Ann. Surg. **151**: 409, 1960.
2. Busch, W.: New Measurements of the Thickness and Weight of the Ventricles in Various Forms of Hypertrophy, With Special Consideration of the Histologic Findings, Arch. Kreislaufforsch. **22**: 267, 1955; Abstr. Circulation **15**: 141, 1957.
3. Moss, A. J., Adams, F. H., O'Loughlin, B. J., and Dixon, W. J.: The Growth of the Normal Aorta and of the Anastomotic Site in Infants Following Surgical Resection of Coarctation of the Aorta, Circulation **19**: 338, 1959.

4. Sauvage, L. R., and Gross, R. E.: Three-Year Results of Autologous Pericardial Grafts in Canine Thoracic Aorta, 1960. (Unpublished data.)
5. Wesolowski, S. A.: Evaluation of Tissue and Prosthetic Vascular Grafts. Monograph to be published by Charles C Thomas, publisher, Springfield, Ill.

DISCUSSION

(PAPERS BY BURMAN [PAGE 635], LONG [PAGE 640], SMYTH [PAGE 653], GILBERT [PAGE 667], TAKARO [PAGE 673], LITTLEFIELD [PAGE 685], WESOLOWSKI [PAGE 692], AND THEIR ASSOCIATES)

DR. CHARLES ROSS, Buffalo, N. Y.—The use of nitrogen mustard in the management of bronchogenic carcinoma has been studied for the last 2½ years by a cooperative group. There were 22 investigators participating in this study.

The nitrogen mustard was given at the time of surgery, initially in a dose of 0.4 mg.; later, when the study became a double blind study, in the amount of 0.3 mg./Kg.

The complications were higher. The mortality rates were identical in the two groups. This slide is a life table analysis of survival, and the 2½ year survival is 30 per cent. There is identical survival in the treated as well as the control group.

So we can surmise that nitrogen mustard used in this fashion will not prolong survival in the surgical treatment of bronchogenic carcinoma.

DR. PAUL NEMIR, JR., Philadelphia, Pa.—It is our opinion too that this method of regional perfusion is a reasonable one in nonresectable carcinoma of the lung, and have had occasion to use it.

[Slide] This slide is of a 60-year-old man with undifferentiated carcinoma of the right lung and mediastinal metastases. We gave this man 0.4 mg./Kg. of nitrogen mustard in 33 ml. with 4 mg. of Renografin, and kept the balloon occluded for a period of 12 minutes. On this film, taken at 7 minutes after the administration of cancericidal agent, you can still see the contrast material in the lung vasculature.

The patient experienced an extreme hot flush on the right side of the chest during the period of occlusion. As soon as the balloon was deflated, he experienced the generalized hot flush that one gets with nitrogen mustard in a single dose. While there may have been slight leakage, we consider this supporting evidence that the cancericidal agent remained fairly well localized for a period of 12 minutes.

It has now been about 3 weeks since the perfusion. We believe that there has been a definite regression in the size of the lesion in the lung, but we have run into the same difficulty that Dr. Smyth reported.

[Slide] This is the chest film taken 48 hours after perfusion. You can see the chemical pneumonitis that has occurred in the right lower lobe. This has been clearing, but what the final status will be, we don't know.

We are in the midst of studying experimentally the effect of cancericidal agents on the vessels. I think that it is encouraging that there has been some decrease in the size of the lesion in the lung.

DR. HARRIS B. SHUMACKER, JR., Indianapolis, Ind.—Although I have had no opportunity clinically to utilize grafts from the right ventricle to the pulmonary artery, I thought you might be interested in an unusual case in which a graft was interpolated between the pulmonary trunk and one main pulmonary artery.

This was a case of an 11-month-old child who had done very badly. He had episodes of intermittent cyanosis and very poor weight gain. During his admission to the hospital he lost weight in spite of the best medical treatment. X-ray studies showed evidence of right ventricular hypertrophy and increase in pulmonary vasculature.

The first catheterization study had to be carried out with continuous administration of oxygen, and was not entirely satisfactory, but a subsequent one was more satisfactory. At the second catheterization the femoral artery saturation was only 52 per cent without

oxygen, but rose to relatively normal levels with administration of oxygen. The pressure in the pulmonary trunk was equal to that in the aortic arch. The left main pulmonary artery arose from the pulmonary trunk, the right from the aortic arch, and there was a large patent ductus with generous angiographic reversal of flow. These anomalies were demonstrated by cine-angiography.

At operation the very large right pulmonary artery was detached from the aortic trunk, and a Dacron graft was interpolated between the side of the pulmonary trunk and the right main pulmonary artery. This went smoothly. It was done as a closed procedure, and the results so far have been very gratifying. Prior to operation the patient weighed 14½ pounds. When seen 5 weeks later, symptoms had disappeared. The child seemed to be quite well, and had gained 4½ pounds during the 5 weeks' time. The child will be re-catheterized within the next few weeks.

This case demonstrates that it is possible to obtain a good result with a plastic prosthesis to the pulmonary artery.

DR. THOMAS C. MOORE, Indianapolis, Ind.—We became interested in the problem of pulmonary artery replacement following the interesting paper which was presented before this Association 2 years ago by Robinson and his associates. Since that time we have replaced the proximal left pulmonary artery in 50 animals, utilizing a variety of grafts. We have used fresh autogenous systemic artery, fresh autogenous systemic vein, and crimped Teflon vascular grafts. The patency in all has been quite satisfactory.

The animals have been observed from 6 to 391 days following operation. Ten of 14 systemic arterial grafts remained patent. Seventeen of 19 venous grafts remained patent, and 16 of 17 Teflon grafts remained patent.

The patency has been encouraging to us, and has been substantiated by our observations with another type of replacement in the superior vena cava, in which we have carried out 78 grafts, using sizes significantly smaller to significantly larger. We have encountered a patency of 86 per cent in the last 50 consecutive grafts. We have also found that all of those which remained patent past an early critical period tended to remain patent.

We also have carried out operations similar to those reported by Dr. Gilbert, employing Teflon grafts from the pulmonary artery to the right ventricle. This has been employed in 12 animals, and the patency has not been quite as satisfactory as his. We have encountered graft occlusion in right ventricular anastomosis in a number of animals.

DR. ADRIAN KANTROWITZ, Brooklyn, N. Y.—During the summer of 1959 I had an opportunity to visit the first thoracic surgery hospital in Moscow, and, subsequent to this, Dr. Kolyesinkov, who is the Director of this unit, sent me a stapling device which was designed to staple the auricular appendage. I thought it might be of some interest to see what this device looks like.

I have a short 2-minute movie which I would like to show. [A film was started.]

The instrument comes in this wooden box, which is plush-lined, and consists of several parts: the stapler itself, a container with two extra magazines, and a small box which contains the tantalum staples. The staples are small and sharpened. They measure about 4 mm. by 4 mm. The two extra magazines are identical, and it comes apart very simply, like this.

First the driving nut is placed on one end of the jaw. This is the staple advance apparatus. The second part of the jaw snaps into position, and the handle just simply clicks into place.

The magazine, containing two lines of eight staples, is simply fixed, on one side of the jaw, and then by simply squeezing the handle the staples are put into position.

Here, demonstrating on a small piece of gauze, the jaws are closed and the staples are driven home. Here is the double row of staples, and this is the obverse side, and on the inside, one can see a nice seal.

This is a patient in which we had just completed doing a commissurotomy. The stapling apparatus is placed just below the Satinsky clamp, and the jaw is advanced, the safety device is opened, and the jaws closed.

Here you can see the double row of staples placed at the base of the left auricular appendage.

DR. KIYOSHI INOKUCHI, Kyushu, Japan.—I am very interested in Dr. Takaro's speech, since several years ago I also worked out a device on the mechanical suture of blood vessels. This involved the multiple stapling of everted vessels with special steel clips. A description of my end-to-end apparatus was published in the *Archives of Surgery* in 1958.

[Slide] Last year I devised a side-to-end apparatus and successfully applied it to 7 patients during splenorenal shunts.

[Slide] Later I constructed another type which is specifically applicable and convenient for end-to-end anastomosis of large vessels. It is of a different type from that described by Dr. Takaro.

[Slide] Some people may be suspicious that the device is only good for laboratory experiments, and that it would be hardly applicable to clinical cases. However, I disagree with such an opinion because of my previous experiences. In long-survival experiments in 135 dogs, there were only three failures from thrombosis. There was none from anastomotic insufficiency.

The results from grafting of a small artery, as shown here, showed only two failures in 34 cases, in contrast to the results from the use of the manual suture, in which there were eight failures in 15 cases.

[Slide] Homologous vein transplantation of the portal vein showed no failures in 9 cases, while there were 4 cases of failure in ten instances when the manual suture was used.

[Slide] So far, I have used my end-to-end stapling device in 22 patients with satisfactory results. Particularly, I should like to point out that the apparatus was also applied to sclerotic, thickened vessels, without many technical difficulties or mishaps.

I believe that a well-designed multiple stapling device can overcome the difficulties in patients with the manual suture, and that it is able to make a good contribution to vascular surgery.

DR. HENRY T. BAHNSON, Baltimore, Md.—We agree with Dr. Littlefield that coronary perfusion is good and necessary. Even when using hypothermia, we continue to use perfusion lest we fall into the same trap as the band wagon of potassium citrate from which most of us have recently alighted.

It is difficult to transfer conclusions from the dog to man, because of the very small size of the right coronary artery. We believe both arteries should be perfused in the human being. This is more trouble [slide] than not perfusing them, obviously.

We have found that the catheters shown here are the easiest of the several types that we have used. We continue to use perfusion cannulas like the one that Dr. Littlefield showed, after the suggestion of Dr. Muller. In most instances, however, this catheter, patterned after one that Dr. Starling used in coronary perfusion many years ago, has worked satisfactorily. These have metal tips on them plus a small balloon and a plastic catheter.

They are relatively easy to insert and are held in with a suture. The balloon is inflated with saline, and when the catheter is removed, this is deflated. We have used them for periods up to 80 minutes with good maintenance of cardiac tone and resumption of cardiac activity when the circulation is restored.

[Slide] We have used this particularly in regard to cusp replacement, although they have also been used in valvuloplasty and removal of calcium from the aortic leaflets. This shows the method of making the cusps which we have used. This is Teflon fabric formed over a mold. When the knitted fabric is heated to 500° F., the plastic assumes the shape of the mold, and one ends up with a cusp like the one shown in the next slide.

[Slide] We have tried to replace all three cusps in a single patient, without success. One of our difficulties has been, I believe, in determining the proper size of these cusps. It

is much simpler when only a single cusp is contemplated, and we believe in many instances, if not most instances, use of a single cusp will probably carry the patient along at least until our technical advances catch up with the patient's disease.

We have used these in 4 patients. With 3 there has been dramatic improvement. The fourth patient died a week later of a myocardial infarction.

The end result looked very much like the silicone-coated cusp which Dr. Hufnagel showed. It was more flexible than the adjacent cusp, on which we had done a valvuloplasty.

DR. CHARLES P. BAILEY, New York, N. Y.—Perfusion of two coronaries is generally better than that of one. However, there is a great variation in human cases, and, in some, the intercoronary collaterals apparently may serve us well, as in cases in which there is difficulty in cannulating the right coronary artery.

[Slide] The right coronary artery sometimes bifurcates almost immediately after its origin, so cannulation may be difficult. This is the equipment we have used, two polyethylene tubes with sponge rubber carrot-like cuffs which are inserted as shown on the next slide.

[Slide] The left coronary cannula is affixed by an encircling purse-string suture placed within the aortic lumen; the right one by a suture passed externally around the right coronary artery. We tried tubing and spring-like devices but have come to not use them. First, they get in the way of the surgeon, and, second, if the aortic incision is made close to the right coronary ostium, a strong assistant holding a metal cannula may pull the coronary artery completely off the aorta. This creates a very difficult problem. [Laughter]

Another thing we have found is that flow rates required in these cases are larger than one would expect. Naturally, these operations are performed for aortic disease, usually stenosis or insufficiency, and the heart is much larger than normal. No doubt the coronary circulation is less adequate than usual. We have found that a flow of 200 or 300 c.c. a minute usually is not enough for an adult at normothermic temperatures and have had to increase it up to 1,000 c.c. per minute in some instances.

Studies on dead hearts in patients of this type, and studies at the operating table, suggest that the desired flow rate is infinitely variable and should be monitored by measuring the pressure within the coronary artery. This may be obtained by direct needle puncture—as distally in the coronary tree, of course, as is feasible. We think the coronary arterial pressure should be kept at about the level of the systemic arterial perfusion pressure. We urge that both coronaries be used whenever possible.

However, if there is a retrograde perfusion flow of dark blood from the right coronary ostium when the left is perfused, that patient probably has good enough intercoronary collaterals to get along, especially if the time of aortotomy is going to be reasonably short.

Announcements

THE AMERICAN ASSOCIATION FOR THORACIC SURGERY

Notice of Annual Meeting

The Forty-first Annual Meeting of The American Association for Thoracic Surgery will be held April 24, 25, and 26, 1961, in Philadelphia, Pennsylvania. Headquarters will be the Sheraton Hotel.

Requests for Hotel Reservations

These should be mailed directly to Mr. Brian Kirby, Reservation Manager, Sheraton Hotel, Philadelphia 3, Pennsylvania. Please mention this Association, the type of accommodation desired, the date, and approximate hour of arrival and departure. If accommodations are desired elsewhere please communicate directly with the hotel of your choice.

Material for the Program

SCIENTIFIC PAPERS.—Abstracts of all papers proposed for presentation at the 1961 meeting *must be received on or before* **November 15, 1960,** otherwise they will not be considered by the Program Committee. These should be labeled "For Thoracic Surgery Forum" or "For Regular Program" depending upon the nature of the subject matter, in the same manner as in previous years. They should contain from 200 to 250 words that accurately reflect the content of the completed paper.

Six, repeat, six clearly legible copies of *each* abstract must be sent to Miss Ada Hanvey, Administrative Assistant, The American Association for Thoracic Surgery, 308 Carondelet Building, 7730 Carondelet Avenue, St. Louis (Clayton) 5, Missouri.

Essayists selected for the program are reminded that papers presented at the meeting *must* be handed to the secretary *immediately* after their presentation. If they are not ready at that time there is a chance that they will not be published in THE JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY.

Applications for Membership

Applications for Associate Membership in the Association must be received by the Membership Committee *not later than* **November 15, 1960,** otherwise the application will be deferred for consideration until the 1962 meeting.

Applicants must be sponsored by three Active or Senior Members of the Association. In addition to the signature on the application form, the sponsors will forward a *separate* letter concerning the applicant directly to:

Frank Gerbode, M.D.
Chairman of the Membership Committee
Presbyterian Medical Center
San Francisco 15, California

Sponsors are reminded that *new* letters of recommendation must be forwarded to the Chairman of the Membership Committee should their applicants be held over for reconsideration a *second year.*